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OF THE NEW YORK ACADEMY OF MEDICINE

Original Articles

by

I. SNAPPER

JOHN S. LOCKWOOD
and
H. T. RANDALL

SECTION ON MICROBIOLOGY

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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

APRIL 1949

MANAGEMENT OF ACUTE RENAL FAILURE*

I. SNAPPER

Physician to the Mount Sinai Hospital and Director of Medical Education

DIETARY TREATMENT

DIETARY treatment is of great importance in all cases of acute anuria not only in acute glomerulonephritis,[1] but also in lower nephron nephrosis.[2, 3, 4] During the last fifty years the treatment of acute nephritis has varied considerably. Until forty years ago every case of acute nephritis was treated with an absolute milk diet. Large quantities of milk, three to four liters daily, were advocated because the fluid allegedly flushed the glomeruli and tubules and kept the urinary passages open. Milk was considered to be especially appropriate for this purpose because it was so bland a nutrient that it could not irritate the kidneys. Finally, the milk diet assured a satisfactory caloric intake. All this changed when in the first decade of the twentieth century Strauss from Berlin and Widal from Paris both demonstrated that in the serum of uremic patients, the non-protein nitrogen and the urea nitrogen content was always increased. A few years later Widal emphasized the importance of the sodium chloride intake for the formation of the edema.

* From the Second Medical Service of the Mount Sinai Hospital, New York City.
Presented October 7, 1948 before the 21st Graduate Fortnight of The New York Academy of Medicine.

As milk contains large amounts of proteins (35 gm. per L.) and sodium chloride (2.5 gm. per L.), both of which were detrimental for patients with acute nephritis, the classical milk diet was abandoned and a diet poor in protein and poor in sodium chloride was advocated. Shortly after the first World War Volhard[5] recommended an absolute starvation diet consisting only of 800 cc. of fruit juice for patients with acute glomerulonephritis. In his opinion recovery of the diseased renal parenchyma required absolute rest and for this purpose not only excretion of chlorides, urea and other substances but also of water had to be restricted as much as possible. After the introduction of this starvation diet cases of anuria and of eclampsia in acute nephritis have become extremely rare.

In the last decade the unfavorable side actions of the starvation diet have been emphasized. Insufficient intake of calories increases the catabolism of body proteins which in its turn leads to an increase of the non-protein nitrogen of the blood. On a starvation diet so much body protein is broken down that from this source alone 20 grams of urea are formed daily. Thus in cases of impaired renal function starvation produces enough urea to cause a rise in the blood urea nitrogen of 20 mg. per cent daily in an adult. This is the reason why many clinicians now attempt to avoid starvation. As proteins and salts should be avoided, Borst[6] has advocated a daily diet consisting of 200 grams of butter and 200 grams of sugar. The butter is first melted, then carefully mixed with the sugar and about 12 grams of flour and some coffee flavor are added. The whole is put into the refrigerator. A teaspoonful of this mixture given in iced condition is palatable, but most patients complain if they have to take 400 grams of this mixture daily. Only patients who have been persuaded to understand the necessity of such a diet may be able to tolerate it. On this diet the body produces only four grams of urea daily. Borst calculates that a patient with no kidney function should live five times as long on such a regime as on a starvation diet. It should also be remembered that toxic substances like potassium which play an important role in uremia, are derived from the endogenous break-down of protein.

Even the Kempner diet[7] (rice, fruit and sugar) consisting of 460 grams of carbohydrates, 20 grams of protein, 0.2 grams of sodium and 0.15 grams of chloride could be used although it contains more protein than the mixture of butter and sugar as advocated by Borst.

After the most serious dangers have been overcome by the butter-sugar or the starvation regime, a diet providing 2,480 calories with four grams of nitrogen, 1,280 mg. of potassium and 470 mg. of phosphorus has been advocated.

Potatoes (boiled)	100 gms.
Rice, Polished (raw)	50 "
Flour (80%)	100 "
Custard powder	25 "
Cream (20%)	200 "
Butter	100 "
Sugar	100 "
Apples or pears	200 "
Vegetables	100 "
Cocoa powder	10 "
Tea infusion	500 "
Coffee infusion	100 "

As far as our own results are concerned, the following figures may be of some importance. Between April 1943 and December 1944, eleven patients with acute nephritis were treated with low salt diet and fluids ad libitum. One patient died. Between December 1944 and August 1948, nine patients with acute nephritis were treated with a starvation diet of 800 cc. of fruit juices for about one week. Thereafter, low salt, low protein diet was given. Of these patients, two died. It is true that nearly all these patients had already been suffering from acute nephritis for several weeks previous to admission to the Hospital. As a rule, no dietary measures at all had been ordered during this period. Under these circumstances, the treatment of the acute nephritis had to start at a stage when in some cases irreparable changes had already occurred. In any case, the difference between the starvation diet and the more liberal low salt, low protein diet do not seem significant.

The introduction of a starvation diet emphasized for the first time the importance of the restriction of the fluid intake in oliguric or anuric patients, irrespective of the etiology of the renal failure. The ingestion of large amounts of fluid in such cases will easily lead to overloading of the circulation with ensuing lung edema and anasarca.[8] A liberal ingestion of sodium chloride will favor the development of these dangerous complications still more. In anuria the fluid intake should not exceed 1,000 cc. per day which is sufficient to balance the normal loss of water through skin and respiratory tract. In case vomiting and diarrhea are present, the water intake should be adequately increased. Ingestion

of NaCl may be necessary if chlorides are lost in the vomitus. Acidosis should be combatted with sodium bicarbonate by mouth. In case intravenous treatment of the acidosis is necessary small amounts of 5 per cent sodium bicarbonate solution are preferable because they contain as much base as large quantities of 1/6 mol. sodium lactate solution. The outlook of anuria whether due to acute nephritis or lower nephron nephrosis will be improved considerably if from the beginning a low sodium chloride, low protein diet with restricted fluid intake or a starvation diet of 800 cc. of orange juice or a butter-sugar diet, is prescribed.

Occasionally cases of oliguria or anuria are encountered where dietary treatment alone does not lead to favorable results. Many such patients are admitted with left heart failure and pulmonary edema because in order to bring about diuresis they have been overloaded with fluid and salt. (Leiter *et al*[8a] Muirhead and Fromm[8b]). Often they are also in severe acidosis or alkalosis. In order to treat such cases methods have been perfected recently by which, at least temporarily, large quantities of the toxic substances which accumulate in the blood during anuria can be removed. These methods consist of continuous dialysis of the blood either by way of an artificial kidney or by continuous lavage of the peritoneal cavity or even of the intestine. Recently exsanguinotransfusion has been recommended. These methods are applied in the hope that by cleansing the blood of the anuric patient life can be prolonged until the tubules have regenerated and diuresis has been restored. The greatest experience has been collected with the artificial kidney as popularized by Kolff[9] and with peritoneal dialysis as revived by Fine, Frank and Seligman.[10] Both methods use the same principle. In the artificial kidney the blood is dialyzed in a cellophane or cellulose-acetate tube; in the peritoneal dialysis the peritoneal membrane is used as a dialyzing membrane. Intestinal irrigation and exsanguinotransfusion are other methods for the treatment of acute anuria which are based upon fundamentally different principles.

Artificial Kidney

History: Abel[11] and his co-workers already in 1913 constructed an artificial kidney. They prevented the coagulation of blood by hirudin and dialyzed the blood by letting it run through celloidin tubes which were submerged into a dialyzing fluid. Haas[12] between 1915 and 1928 followed up these experiments using celloidin tubes. He also started

with hirudin but soon recognized that for the dialysis of human blood, heparin had to be used. He actually dialyzed the blood of two uremic patients. Necheles[13] in 1923 used tubes made of peritoneal membrane and Thalhimer[14] in 1938 cellophane tubes. Both these investigators performed dog experiments only. The first apparatus which could be readily used on patients was constructed by Kolff in 1943.[9] He could obtain practical results because he could use 1.) Cellophane or the closely connected cellulose acetate (Visking), 2.) Heparin, 3.) A large dialyzing surface, 4.) A dialyzing fluid of correct composition.

Table I shows the differences between the Kolff kidney and its predecessors. Slight modifications of Kolff's apparatus have been published.[15, 16] Other artificial kidneys have been constructed[17] and the models devised by Murray[18] and by Alwall[19] have actually been used in humans (Table I).

Different investigators used dialyzing solutions of different composition. As brought out by Abel plasma only contains 0.6 per cent of chloride and 0.9 per cent of sodium. If the bathwater contains more chloride than the plasma absorption of excessive amounts of chloride ions takes place. This explains why the use of 0.9 per cent sodium chloride as the dialyzing fluid easily leads to anasarca and pulmonary edema. Kolff's dialyzing fluid (Table II) contains 0.6 per cent NaCl, 0.4 per cent KCl, 0.2 per cent of $NaHCO_3$, and 1.5 to 2 per cent of glucose, that is, 383 mg. per cent of chloride (plasma water 370 to 420 mg. per cent), 291 mg. per cent Na (plasma water 340 to 360 mg. per cent), 21 mg. per cent K (plasma water 18 to 22 mg. per cent), and 53 volume per cent of CO_2 (plasma water 55 to 77 volume per cent). The bathwater does not contain calcium because this would be precipitated by the sodium bicarbonate. It follows that during the dialysis in the artificial kidney the blood loses considerable amounts of calcium and calcium gluconate must be repeatedly injected intravenously during the procedure.

Glucose is added in order to make up for the osmotic pressure of the plasma proteins. In patients with edema the glucose withdraws fluid from the blood plasma. At the same time glucose absorbed from the dialyzing fluid into the blood has a definite caloric value. On the other hand, the blood sugar of the patients rises to very high values and in the future this excess of glucose may have to be replaced by a non-dialyzable colloid substance. Until now this has not been possible.

TABLE I—HISTORY OF THE DEVELOPMENT OF THE ARTIFICIAL KIDNEY

Investigator	*Material*	*Surface*	*Anticoagulant*	*Capacity*
Abel et al.[11] 1913	Celloidin	3,200 sq.cm.	Hirudin	20 gms. NPN in 112 hrs.
Haas[12] 1915-28	Celloidin	2,160 sq.cm.	Hirudin Heparin	2.7 gms. NPN in 7 hrs.
Necheles[13] 1923	Tubes of peritoneal membrane	4,000 sq.cm.	Hirudin	
Thalhimer[14] 1938	Cellophane	4 tubes of 2 x 30 cm.	Heparin	200-700 mgms. BUN in 3-5 hrs.
Kolff[9] 1943-44	Cellulose (Viking)	33-50 yds. 20,000 sq.cm.	Heparin	42-141 gms. BUN in 8 hrs.
Murray[18] 1947	Cellulose (Viking)	12 yds.	Heparin	6.6 gms. NPN in 8 hrs. 49 gms. NPN in 26 hrs.

TABLE II—COMPARISON OF ELECTROLYTES IN PLASMA AND DIALYZING FLUIDS

	Plasma Water	*Kolff[9] Artificial Kidney*	*Kolff & Kop Peritoneal Dialysis[22]*	*Tyrode Solution*
Na mg.%	313-330	291	291	343
Cl mg.%	340-393	383	383	502
K mg.%	16-20	21	21	11
CO_2 vol.%	55-77	53	53	27
Ca mg.%	10	—	10	7
Glucose mg.%	100	2,000	2,000	100 plus traces of P and Mg.

Whereas Bywaters and Joekes used the dialyzing solution as recommended by Kolff, Murray dialyzed against a solution containing 0.8 per cent of NaCl, 0.02 per cent of KCl, 0.02 per cent of $CaCl_2$, 0.01 per cent of $MGCl_2$, 0.1 per cent of $NaHCO_3$ and 0.1 per cent of glucose. Theoretically this solution contains an excess of chloride ions (Table II). Alwall used 0.9 per cent NaCl as dialyzing fluid.

Results: Kolff has treated thirty-one patients, five of whom survived. Two of the five survivors suffered from acute glomerulonephritis, three from lower nephron nephrosis (hepato-renal syndrome after cholecystitis, mercury poisoning and sulfa-drug anuria). In his opinion, dialysis in the artificial kidney is indicated if the blood urea nitrogen has increased to 160 mg. per cent or if the potassium content of the blood has gone up or if severe acidosis has developed. The dialysis lasts usually between five and fourteen hours. During this period so much urea is removed from the blood that the blood urea nitrogen goes down to 50 or 60 mg. per cent. At the same time considerable quantities of creatinine, uric acid, indican, xanthoprotein products are cleared from the blood and can be recovered from the bathwater.

Two of the ten patients treated by Bywaters and Joekes[15] survived. One patient developed oliguria after an explosion and the urea nitrogen of the blood had gone up to 180 mg. per cent. Three days after the dialysis, diuresis started. The second patient became severely uremic after an operation under myanesin anesthesia, probably due to hemolysis caused by the anesthetic. In this patient uremia developed, although he excreted daily about one liter of urine. On the 26th day after operation the blood urea nitrogen had risen to 200 mg. per cent and the artificial kidney was used. Nine days after dialysis, the urinary output increased considerably and the patient recovered. Two of the patients treated with Murray's artificial kidney survived.[18] The first one was a woman of 26 years who after an abortion developed anuria. On the ninth day the patient was treated with the artificial kidney for one hour, two days later for eight hours and again three days later for seven hours. The patient improved and two days later diuresis set in. The other patient, 40 years old, received a transfusion with incompatible blood. After nine days of anuria she was deeply uremic. Treatment with the artificial kidney for 6 hours resulted in a great improvement. Next day diuresis started and the patient recovered. Table III gives a review of the reported cases of recovery after dialysis in the artificial kidney.

TABLE III—CASES RECOVERED AFTER DIALYSIS IN ARTIFICIAL KIDNEY RECORDED IN THE LITERATURE

Diagnosis	*Anuria*	*Dialysis*	*Blood Urea Nitrogen Mg.%*		*Diuresis After Dialysis*
			Before	*After*	
Hepatorenal syndrome[9]	8 days	111 hrs. 80 L 60 gm.U+	190	58	2 days
13 yrs. Acute Nephrosis[8]	7 days	4½ hrs. 34 L 45 gm.U+	120	65	1 day
54 yrs. Acute Nephrosis[9]	5 days oliguria	5 yrs. 60 L 66 gm.U+	150	80	2 days
50 yrs. Sulfa drugs[9]	4 days	51 L 52 gm.U+	100	49	2 days
23 yrs. $HgCl_2$[9]	7 days	39 gm.U+	174	69	3 days
31 yrs. Crush syndrome[15]	8 days 150 cc.	5 hrs. 21 L 32 gm.U+	180	100	3 days
52 yrs. Postoperative (myanesin hemolysis)[13]	26 days (output 1 L daily)	41 hrs. 19 L 38 gm.U+	200	120	9 days
26 yrs. Abortion[12]	9 days (output 35 cc. daily)	1.8, 7 hrs. 6.7 gm. NPN	120 NPN	65 NPN	3 days
40 yrs. Transfusion reaction[12]	8 days	26 hrs. 49.4 gm. NPN	179 NPN	100 NPN	1 day

TABLE IV—TWO MOUNT SINAI HOSPITAL CASES RECOVERED AFTER DIALYSIS IN ARTIFICIAL KIDNEY[20]

Diagnosis	*Anuria*	*Dialysis*	*Blood Urea Nitrogen Mg.%*		*Diuresis After Dialysis*
			Before	*After*	
25 yrs. $HgCl_2$	5 days	6 hrs. 50 gm. U+	150	70	7 days
		1st dialysis			
30 yrs. CCl_4	9 days	6 hrs. 34 gm. U+	108	54	
		2nd dialysis			
	15 days	6 hrs. 41 gm. U+	85	35	2 days after 2nd dialysis

Alwall has treated five patients, three with acute and chronic nephritis, one with polycystic kidney and one with a carcinomatous obstruction of the ureters. In all patients the urea and other nitrogenous waste products which had accumulated in the blood were reduced considerably. All felt better after the dialysis but they died shortly thereafter from the original disease.

At the Mount Sinai Hospital six patients with anuria have been treated with the Kolff kidney (Fishman *et al*[20]). Four were in the last stages of uremia and died shortly after the dialysis. The two last patients were in better shape before the treatment started and recovered after dialysis (Table IV).

The first patient was a woman, 25 years of age, who had introduced 2.5 grams of mercury bichloride in tablets into the vagina. She was admitted on the fifth day of the anuria and only then was BAL treatment started. She had extensive necrosis of the mucous membranes of the mouth and vagina. The hemoglobin had gone down to 8.5 grams and the general condition was very poor indeed. The blood serum contained large amounts of urea and other nitrogenous products, the carbon dioxide combining power was 26 volume per cent.

On the night of admission dialysis with the artificial kidney was performed. The injection of 250 mg. of heparin proved to be sufficient to keep the clotting time of the blood between one and four hours during the six hours the dialysis lasted. The dialysis resulted in the removal of 24 grams of urea nitrogen, 6.7 grams of uric acid, 9.7 grams of creatinine and 6.5 grams of protein. At the end of the dialysis the nonprotein nitrogen of the blood had diminished from 150 mg. per cent to 70 mg. per cent, the urea nitrogen from 116 mg. per cent to 30 mg. per cent, the uric acid from 14 mg. per cent to 6.9 mg. per cent, the creatinine from 14.1 to 5.7 mg. per cent, the inorganic

phosphorus from 7 to 1.5 mg. per cent. During the dialysis the general condition of the patient improved considerably. Nevertheless, the following days were critical because of severe vomiting and diarrhea. The urea nitrogen rose again notwithstanding a gradual increase of the urinary output. On the seventh day after dialysis the quantity of urine approximated the intake of fluid. However, only when the diuresis increased to several thousand cc. and the urea nitrogen concentration of the urine approached 400 mg. per cent, did the nitrogenous retention products begin to disappear from the blood. During this period a diet high in carbohydrate, high in fat and with extreme limitation of protein was given. The intake of fluid was restricted to about one liter for every 24 hours. On an average daily approximately 4 grams of sodium chloride and 16 grams of sodium bicarbonate were given by mouth, depending upon the chloride content and the carbon dioxide combining power of the serum. Ultimately, the renal function returned to normal.

The second patient was a man of 30 years who for five hours had been exposed to fumes of carbon tetrachloride. Seven days after the intoxication the patient became completely anuric. On the eighth day he was admitted to the Mount Sinai Hospital. He was severely uremic, with high nonprotein nitrogen (170 mg. per cent) and acidosis (carbon dioxide combining power of 28 volume per cent). On the second day after admission the patient was treated with the artificial kidney for six hours. The injection of 250 mg. of heparin resulted in a satisfactory retardation of the clotting. During the dialysis 34 grams of urea, 3.8 grams of uric acid, 12.4 grams of creatinine, 4.1 grams of inorganic phosphorus and 8.3 grams of protein were removed. The blood urea nitrogen came down from 108 mg. per cent to 54 mg. per cent, the uric acid from 11.3 mg. per cent to 8.3 mg. per cent, the inorganic phosphorus from 4.1 mg. per cent to 3.1 mg. per cent. Extreme oliguria persisted and the nitrogenous waste products accumulated again in the blood. Six days after the first dialysis he was treated for the second time with the artificial kidney for six hours. During the dialysis 41 grams of urea, 3 grams of uric acid, 16.7 grams of creatinine, 2.7 grams of inorganic phosphorus and 22 grams of protein were removed. The blood urea nitrogen came down from 85 mg. per cent to 35 mg. per cent, the uric acid from 5.6 mg. per cent to 2.5 mg. per cent, the creatinine from 27.2 mg. per cent to 19.8 mg. per cent, the inorganic phosphorus from 7.5 mg. per cent to 3 mg. per cent. Two days later, diuresis set in which resulted in a gradual recovery.

The clinical course in this case was characterized by freedom from uremic manifestations despite a long period of renal insufficiency and the accumulation of large amounts of urea and other retention products in the serum.

Everytime a patient with anuria and uremia recovers after treatment with the artificial kidney, the question can be raised as to whether this patient would not have recovered spontaneously. This holds true especially for patients who are no longer completely anuric and where oliguria has already replaced the anuria. During the first weeks after diuresis has started, retention of urea and other catabolic products in the blood may still be progressive. As long as the urea concentration in the urine is less than 400 mg. per cent the urea nitrogen and the non-protein nitrogen of the blood continue to increase. It may be advisable to dialyze such an oliguric patient in order to relieve the blood from the excess of waste products, but in such cases the artificial kidney can hardly be considered to have acted as a life-saving device. The difficul-

TABLE V—PERITONEAL DIALYSIS

		Animal Experiment	
1923	Putnam	Cats	Intermittent
1923	Ganter	Guinea pigs	Intermittent
1925	Lanzberg & Gnoinski	Guinea pigs	Intermittent
1926	Rosenak & Siwon[21]	Dogs	Continuous
1927	Heusser & Werder[23]	Dogs	Continuous
1932	Bliss, Kastler & Nadler	Dogs	Intermittent
1932	Von Jeney	Dogs	Continuous
1932	Von Haam & Fine	Rabbits	Intermittent
1946	Abbott & Shea	Dogs	Intermittent
1946	Seligman, Frank & Fine	Dogs	Continuous

ties encountered in the appraisal of such cases are clearly demonstrated by the following patient.

Three days after exposure to carbon tetrachloride a patient developed anuria. After the anuria had lasted for three days, diuresis started. Unfortunately, he then was treated with large amounts of fluid and saline so that when ten days after the intoxication he was admitted to The Mount Sinai Hospital, left heart failure had set in. In the Hospital the daily diuresis gradually increased. On the first day of admission it was only 260 cc., on the sixth day of admission, that is, 16 days after the intoxication, it had risen to 1605 cc. Notwithstanding this apparently satisfactory output, the urea nitrogen of the blood had increased progressively to 212 mg. per cent. Patient was irrational and heart failure still persisted. The outlook seemed hopeless. On the night of the 16th day, the patient would have been dialyzed were it not that the artificial kidney was out of order. In the next days the diuresis increased rapidly and the patient recovered spontaneously. Nine days later the blood urea nitrogen was only 28 mg. per cent.

Summarizing it must be pointed out that no certain conclusion can be reached as to whether dialysis by the artificial kidney is actually a life-saving device. At the same time, in the eleven patients who recovered the artificial kidney seems to have had a favorable influence upon the course of the disease. Thus the circumstantial evidence appears

to be in favor of the opinion that dialysis in the artificial kidney results in temporary improvement which may perhaps make survival possible until restoration of the renal function sets in.

Peritoneal Dialysis

History: As in a real dialyzer substances travel from the peritoneal cavity to the blood and in the reverse direction, with the exception of colloids. Table V shows that the method of peritoneal dialysis has been developed gradually and is based upon a long series of experiments. The first experiments with continuous peritoneal dialysis were done by Rosenak and Siwon.[21] After intraperitoneal injection of a solution which is similar in ionic concentration and in osmotic pressure to plasma, no change in the composition of either the peritoneal fluid or plasma occurs. Solutions used for peritoneal dialysis should therefore contain not more than 0.6 per cent of sodium chloride (Table VI).

Seligman, Frank and Fine[10] calculated from their dog experiments that if 1.8 to 3 liters of fluid flow through the abdominal cavity per hour, a urea clearance of 12 to 16 cubic centimeters of blood per minute is obtained. Later Kolff and Kop[22] found in humans that when the inflow of the dialyzing fluid into the peritoneum is kept at one liter per hour, a urea clearance of eleven centimeters of blood per minute results. These figures agree very satisfactorily. It follows that removal of catabolic products from the blood by peritoneal dialysis is equivalent to 40 to 75 per cent of the clearing capacity of the kidneys.

The amount of urea and nitrogen derivatives which can be removed from the blood of uremic patients by peritoneal dialysis of three days duration varies between 20 and 60 grams. In one case Kolff and Kop even removed 300 grams of urea. During the dialysis the urea content of the dialyzing fluid goes up to about 50 per cent of the urea content of the blood.

Results: Table VII shows the increase of popularity of peritoneal dialysis as a clinical method in this part of the world since the publications of Seligman, Frank and Fine.[10]

In 1927 Heusser and Werder[23] reported experiments with intraperitoneal dialysis on dogs. In the same article they mention briefly, in passing, that they also treated three uremic patients with continuous peritoneal dialysis. The results were not favorable evidently, because not enough fluid passed through the peritoneal cavity.

TABLE VI

	Cunningham and Darrow	*Frank, Fine and Seligman (Tyrode[10])*	*Hartmann*	*Abbott and Shea "A" solution*	*Odel and Ferris and Pearson[27] "P" solution*	*Kolff Artificial Kidney*	*Kolff and Kop:[22] peritoneal dialysis*
NaCl Mg. %	650	800	600	610	600	600	600
$NaHCO_3$ Mg. %	250	100		220	300	200	200
KCl Mg. %	18	20	40	35	20	40	40
$CaCl_2$ Mg. %		10	20	23	10		28
NaH_2PO_4 Mg. %		5		7	5		
$MgCl_2$ Mg. %		10		5	10		
Glucose %		1½		1-2	2	1-3	1-3
Natrium-lactate			240				

Different solutions used for peritoneal dialysis

TABLE VII—PERITONEAL DIALYSIS IN HUMANS

		Cases	*Survived*
1927	Heusser and Werder[23]	3	0
1934	Balasz and Rosenak[24]	2	0
1938	Rhoads[25]	2	0
1938	Wear, Sisk and Trinkle[26]	5	1
1946	Fine, Frank and Seligman[10]	4	1
1946-1948		64	19
	Total	80	21

In 1934 together Balázs and Rosenak[24] published the first complete observations on continuous peritoneal irrigation in two patients with fatal uremia, due to mercury poisoning. In the first case the dialysis lasted only for thirty minutes during which time the peritoneum was perfused with 12 liters 4.2 per cent glucose solution. Only a few grams of nitrogen were removed. The same holds true in the second case where 19 liters of 0.8 per cent sodium chloride solution were used as dialyzing fluid.

Rhoads[25] in 1938 treated two patients with chronic nephritis in the same way. He removed large amounts of urea. The patients reacted favorably but died ultimately from their disease. Wear, Sisk and Trinkle[26] in 1938 treated five patients with continuous peritoneal dialysis. One patient who was uremic and had a stone in the urinary bladder survived. In his case peritoneal dialysis with Locke solution resulted in the removal of 16.4 grams of urea. The non-protein nitrogen of the serum came down considerably, the patient could be operated and recovered. Thus previous to the publication of Fine and associates, twelve patients had been treated with peritoneal dialysis of whom one[26] recovered. Four patients were treated by Seligman, Frank and Fine.[10] One patient with anuria after sulfathiazole medication recovered. This was probably the first patient where the possibility that peritoneal dialysis had worked as a life-saving device could be considered. Since their article more than 68 patients have been treated of whom nineteen recovered.[22, 27-37] Twenty-one of the eighty-one cases published were treated by Kolff and Kop with five survivors.[22] Table VIII mentions the cause of anuria in the patients who survived. Table IX illustrates that success has been obtained even in cases where the composition of the dialyzing fluid was not optimal.

Comparison between the artificial kidney and peritoneal dialysis shows that both methods have their advantages and disadvantages. Heparinization necessary for treatment with the artificial kidney may cause disagreeable hemorrhages during and after the treatment. This danger is greatly decreased since protamine sulphate has become available. Peritoneal dialysis cannot be used immediately after an abdominal operation or when extensive adhesions or peritonitis are present. The artificial kidney is a complicated apparatus which requires adept handling by an expert team whereas the instruments necessary for peritoneal dialysis are simple.

TABLE VIII—RECOVERY AFTER PERITONEAL DIALYSIS

Transfusion reactions	5
Mercury intoxication	4
Sulfathiazole	2
Anuria due to other causes (hyperemesis, prostatism, etc.)	10

TABLE IX—SOLUTIONS USED FOR PERITONEAL DIALYSIS IN 19 RECOVERED PATIENTS

Kolff and Kop solution	5
Tyrode solution	6
Hartmann solution	3
A. solution	2
P. solution	1
0.9% NaCl	1
Locke solution	1
1.8% NaCl	1
Different solutions	1

The fact that Kolff and Kop also used the artificial kidney in six of the twenty-one patients whom they treated with peritoneal dialysis shows that the indications for both methods often run parallel (blood urea nitrogen of about 180 mg. per cent, increased potassium content of the serum or marked acidosis). Other investigators start dialysis in patients with mercury poisoning when the anuria exists for three days. Clinical experience has shown that in such cases the outlook is very serious indeed, even if the urea nitrogen content of the blood has not yet reached a level of 180 mg. per cent.

In every patient with uremia due to anuria who recovers after peritoneal dialysis, the possibility that recovery could have been a spontaneous one has to be considered. In addition the dangers and complications of this method should not be underestimated. Peritoneal infection, difficulties in maintaining a satisfactory flow through the peritoneal cavity,[38] abdominal colic and meteorism are frequently the reason why a dialysis has to be terminated prematurely.

Nevertheless, in some of the twenty patients who recovered after

peritoneal dialysis the method apparently has had a favorable influence upon the course of the disease.

Intestinal Irrigation

Introduction: It is true that by irrigation of the intestine, especially of the small intestine, nitrogenous products can be removed from the blood. However, it seems hardly appropriate to designate intestinal irrigation as intestinal dialysis. Intestinal irrigation cannot be identified with dialysis in the artificial kidney or through the peritoneal membrane. If the dialyzing fluid in the artificial kidney or in the peritoneum is isotonic and isoionic with the plasma, then the quantity of fluid and electrolytes in the artificial kidney or in the peritoneal cavity does not, or hardly changes. In contrast, the intestinal wall has specific absorbing qualities and even from an isotonic and isoionic solution large quantities of salts and water are absorbed. The liberal absorption of water and salts from the intestine irrespective of the composition of the irrigation fluid, is a disadvantage in uremic patients with anuria or oliguria, because it easily leads to anasarca and lung edema. Only a relatively small amount of irrigation fluid can be recovered after intestinal irrigation with isotonic fluids, especially when the irrigating fluid runs in slowly. Thus the concentration of the urea present in the fluid recovered from the intestine is high but the total amount of urea removed is only small.

In order to avoid these disadvantages an isotonic solution of 5 per cent magnesium sulphate has been used for intestinal irrigation in animal experiments. As magnesium sulphate is not, or is hardly absorbed from the intestinal lumen, irrigation with this solution will not lead to absorption of excessive amounts of water or salts. In addition, old experiments (Hamburger[39]) seem to indicate that the resorptive power of the intestinal epithelium is markedly decreased after it has been in contact with magnesium sulphate. However, the question may be raised whether large amounts of magnesium sulphate even in isotonic solution will have an irritating effect upon the intestinal wall. Most investigators have added at least a trace of a magnesium salt to the irrigation fluid.

Irrigation of the Colon: Landsberg and Szenkier[40] in 1930 experimented on the influence of colonic irrigation on the condition of rabbits made uremic by uranium nitrate. They found that after lavage of the colon through an appendicostomy tube about 20 mg. per cent urea nitrogen was present in the irrigation fluid. The blood urea nitro-

TABLE X—COLONIC IRRIGATION

	Irrigation fluid	*B.U.N.*
Landsberg and Szenkier	U.N. 20 mg.%	Unchanged (rabbits)
Kolf: Case 1	U.N. 18 mg.%	60 mg.%
Case 2	U.N. 5 mg.%	190 mg.%
Daugherty, *et al.*	U.N. 7 mg.%	180 mg.% before 118 mg.% after
Creatinine unchanged. Hyperchloremic acidosis and polyuria developed		
Removed by irrigation " in urine	1.66 gm. N.P.N. 19.6 mg. N.P.N. } 6 hrs.	

gen of the rabbits, however, did not decrease after colonic irrigation. Pendleton and West[41] who experimented mainly with the removal of urea from the blood by irrigation of the small intestine felt that the colon could also be used for the same purpose.

The evidence available indicates that in patients with uremia, irrigation of the colon does not lead to the removal of significant amounts of urea and other nitrogenous products (Table X). Kolff[9] performed colonic irrigation through an appendicostomy. In the first patient thin feces leaked through the anus and further lavage had to be given up. The urea nitrogen content in the escaping perfusion fluid was 18 mg. per cent as compared with a blood urea nitrogen of 60 mg. per cent. In the second patient the thick tube placed in the rectum was closed off by the sphincter ani, and in this way loss of the perfusion fluid was prevented. Although the blood urea nitrogen was 190 mg. per cent, the perfusion fluid only contained 5 mg. per cent nitrogen. Daugherty and associates[42] also perfused the colon in one patient with uremia through an appendicostomy. The perfusion lasted 64 hours. During this period first 35 liters of P-solution (Table VI), later 5½ liters of a P-solution with reduced sodium chloride (480 mg. per cent), and increased $NaHCO_3$ content (350 mg. per cent) were used. After the irrigation the patient's weight had increased by 6.25 pounds. The serum chloride rose from 92.3 to 118.5 milliequivalent, the carbon dioxide combining power decreased from 22.8 to 15.4 milliequivalent. Thus during the colonic irrigation hyperchloremic acidosis developed and

the experiment had to be stopped because anasarca and lung edema set in. At the same time the amount of nitrogen removed was very small. In 64 hours, 24.5 liters of irrigation fluid could be collected containing in total, 1.66 grams of non-protein nitrogen (7 mg. per cent). The blood urea nitrogen came down from 180 mg. per cent to 112 mg. per cent after irrigation, but the creatinine content of the serum (8-9 mg. per cent) did not change. The decrease of the urea nitrogen content of the blood may have been secondary to the polyuria which set in during colonic irrigation, evidently caused by the absorption of large amounts of fluid from the colon. During the colonic lavage 8.16 liters of urine containing 19.6 grams of non-protein nitrogen were voided. This figure is in sharp contrast to the 1.66 grams removed by the colonic irrigation. The renal elimination of nitrogen even by diseased kidneys exceeds more than ten times the removal of nitrogen compounds by colonic irrigation.

Irrigation of the Small Intestine: Irrigation of the small intestine seems to have given somewhat more encouraging results.

Pendleton and West[41] in 1932 inserted a rubber tube into the middle third of the duodenum and another large tube into the ileum near the ileocecal junction (Table XI). The bowel above and below the two tubes was tied off. Fluids were run in through the proximal tube and left the small bowel through the distal tube.

When normal saline was placed in the small bowel of normal dogs the urea content of the intestinal contents rapidly rose to levels which sometimes even exceeded slightly the blood urea nitrogen. Then nephrectomized dogs were injected intravenously with urea in order to obtain a rapid rise of the urea in the blood. In one dog the urea nitrogen of the blood went up to 265 gram per cent. Five minutes after the introduction of saline into the small bowel, the urea nitrogen of the intestinal content had risen to 25 mg. per cent, after fifteen minutes it was 153 mg. per cent. Two hours after the injection the urea nitrogen of the blood was 290 mg. per cent, of the solution in the intestine 299 mg. per cent. After three hours the blood urea nitrogen had fallen to 245 mg. per cent while the bowel still contained 281 mg. per cent. In order to prevent the absorption of large quantities of water Pendleton and West later used 5 per cent magnesium sulphate solution as irrigation fluid. The urea passed quickly from the blood to the magnesium sulphate solution in the intestine and after a short time the urea of the

TABLE XI—IRRIGATION SMALL INTESTINE IN DOGS

	In Irrigation Fluid	*B.U.N. mg.%*		*Time*	
		Before	*After*		
Pendleton and West	280-290 mg.% U.N.	299	245	3 hrs.	Dogs NaCl 9.9%
Rogers *et al.*	4.3-5.4 gm. N.P.N.	198	126	6 hrs.	
		190	112		
		231	145		
Seligman *et al.*	Loop of 10 feet—10% of maximal renal clearance				

blood and bowel contents were equal. After introduction of the magnesium sulphate solution a constant fluid volume was maintained in the intestine. In these animal experiments the magnesium sulphate did not cause any difficulties from bowel irritation.

Fine, Frank and Seligman[10] performed continuous irrigation of an isolated loop of ileum, but could remove only small amounts of urea. They calculated that continuous perfusion of a loop of small intestine of 10 feet long would be necessary in order to obtain a urea clearance of 7.5 cc. of blood per minute, that is, about 10 per cent of the maximal normal renal clearance.

Rogers, Sellers and Gornall[43] placed a thin triple bore rubber tube with a small balloon on the tip in the small intestine of dogs (Table XI). They had to manipulate the tube into position through an abdominal incision. Warm physiological saline was introduced above the inflated balloon and withdrawn through another opening of the same tube several feet higher up. Using 12 to 18 liters of perfusion fluid over a period of about six hours, they were able to reduce the non-protein nitrogen of the serum of nephrectomized dogs considerably. In one case the azotemia was lowered from 198 to 126 mg. per cent. In two other cases from 198 to 112 mg. per cent and from 231 to 145 mg. per cent respectively. The rinsing fluid after perfusion contained 4.3 to 5.4 grams of non-protein nitrogen.

The literature mentions the following patients in whom uremic conditions were treated with intestinal irrigation (Table XII). Kolff[9] irrigated an isolated loop of ileum 40 inches long in a patient with uremia. Each end of the loop was connected with the outside by an

TABLE XII—IRRIGATION SMALL INTESTINE IN UREMIA

	In Irrigation Fluid	*B.U.N. mg.%*			
		Before	*After*	*Time*	*Irrigation Fluid*
Kolff	2.3 gm. U.N.	Unchanged		10 h.	Kolff solution
Daugherty *et al.*	4 gm. U.N. in 24 h. (10 mg.%)	155	130	24 h.	P-solution
			150	48 h.	
		(creatinine unchanged)			
Oppenheimer and Rosenak	70 & 110 mg.% U.N.	90	83	5 h.	Modified
		83	46	18 h.	Tyrode solution
Marquis and Schnell		330	121	12 h.	NaCl 0.9% or
			66	24 h.	Glucose 10% or NaCl 0.8% plus $NaHCO_3$ 0.1%
	Creatinine unchanged	49		7 d.	

NaCl 0.9%:—Edema and ascites
Glucose 10%:—acidosis
NaCl 0.8% and $NaHCO_3$ 0.1%—hypokaliemia

ileostomy. The continuity of the remaining ileum was restored by an end to end anastomosis. Infection, followed by cicatrization of both stomas of the loop, rendered perfusion of the isolated loop difficult and lavage was only occasionally successful. The best result was obtained when eight liters of fluid passed through the loop in the course of ten hours. During this time 2.3 grams of urea nitrogen were removed. When the lavage fluid runs in very slowly the return flow is necessarily very small. Under these circumstances, the urea nitrogen of the irrigation fluid may go up to 100 mg. per cent. If the perfusion rate is kept to one liter per hour, about 200 mg. of urea nitrogen can be removed per hour. There is probably no advantage in letting the fluid run much faster. It follows that if irrigation during 24 hours would have been possible, nearly 5 grams of urea nitrogen could have been removed every day. This, together with a diet of only fats and carbohydrates, might well keep a nephritic patient at least temporarily in balance. However, the difficulties of perfusion of the ileum loop in Kolff's patient were so great that definite conclusions could not be reached. Kolff used as perfusion fluid the same solution as he used for dialysis with the artificial kidney (Table VI).

Other clinicians have tried to irrigate the small intestine by using

Miller-Abbott tubes or comparable instruments. It is evident that nausea and vomiting as regularly seen in uremia must be a great hindrance to the intra-nasal introduction of Miller-Abbott tubes. A return tube, small enough to be passed by oral or nasal route will usually be too thin to collect large amounts of the irrigated fluid. Thus, too much fluid remains in the bowel and leads to diarrhea. Repeatedly an appendicostomy has been performed through which the irrigation fluid could be sucked out by a pump. The capacity of an appendicostomy tube is not sufficient to cope with large amounts of irrigation fluid and Daugherty and associates are even suggesting that a cecostomy be performed for this purpose.

Daugherty[42] performed intestinal irrigation in a patient with chronic nephritis. The fluid was introduced via a nasal tube which was pushed into the duodenum. At the same time an appendicostomy was performed through which a tube connected with a suction pump was introduced. In this case, also, the suction through the appendicostomy was insufficient and profuse watery stools were produced. During twelve hours of intestinal irrigation 2,750 cc. of fluid were recovered through a rectal tube and only 300 cc. through the appendicostomy tube. The intestinal irrigation was continued for two days at the end of which the patient died. The urea nitrogen of the serum at the beginning of the experiment was 155 mg. per cent, after one day of irrigation it was 130 mg. per cent, and after two days it was again 150 mg. per cent. The creatinine of the serum was high and did not change, the chloride content of the serum rose slightly from subnormal to normal values. The carbon dioxide combining power remained about the same. In 2,010 cc. of fluid recovered from the rectum 10 mg. per cent of urea nitrogen was found.

Oppenheimer and Rosenak[44] passed a modified Miller-Abbott tube through the nose until it reached the middle part of the small intestine. Over a period of five hours four gallons of a solution containing NaCl 0.669 per cent, KCl 0.004 per cent, $CaCl_2$ 0.013 per cent, Mg. lactate 0.0065 per cent, acid sodium phosphate 0.0005 per cent, sodium bicarbonate 0.15 per cent and glucose 1.5 per cent were introduced. The next day six gallons were given in the course of 18 hours. During this procedure the blood urea nitrogen went down from 90 mg. per cent to 46 mg. per cent. The recovered fluid contained 111 and 70 mg. per cent of non-protein nitrogen. They also experienced difficulty in re-

covering the irrigation solution because too much was absorbed. This may explain the relatively high concentration of the nitrogen content of the fluid recovered. At the same time the patient suffered from diffuse watery diarrhea.

Marquis and Schnell[45] reported a case where at least the technical part of the intestinal irrigation was efficient. After two days of anuria due to ingestion of cleaning fluid the patient became completely comatose. Non-protein nitrogen had risen to 330 mg. per cent. At this time two Miller-Abbott tubes were placed in the small intestine, one in the third portion of the duodenum and the other in the lower part of the ileum. In twelve hours 22 liters of fluid were run slowly through and the non-protein nitrogen of the serum dropped to 121 mg. per cent, twelve hours later it was 66 mg. per cent. In the fluid recovered the non-protein nitrogen went up to 50 mg. per cent. Intestinal irrigation was continued for seven days. Gradually diuresis set in and the intestinal irrigation was continued on a half-time schedule. Ultimately it was discontinued. In the next forty-eight hours the urea nitrogen of the blood rose rapidly from 49 to 103 mg. per cent, and the intestinal irrigation was reinstituted. On the tenth day of intestinal irrigation the patient suddenly died.

During the first twenty-four hours of irrigation with 42.7 liters of normal saline, six liters of fluid were retained and general anasarca set in. Thereafter, 20 per cent glucose was used instead of saline. The edema, ascites and hyperpnea disappeared, but acidosis set in. Ultimately 0.8 per cent sodium chloride combined with 0.1 per cent sodium bicarbonate was used. The decrease of the urea nitrogen of the blood indicates that in this way large amounts of urea were removed. As in the other cases of intestinal irrigation the creatinine content of the serum remained high and did not change. The cause of death in this case may have been due to the removal of large amounts of potassium during the irrigation. On the day before death there were many extra systoles. The potassium content of the serum was determined but the result was reported after the death of the patient. The potassium content was found to be 4.6 mg. per cent instead of 16-19 mg. per cent and the patient may well have died from ventricular fibrillation.

It seems that this is the only case where intestinal irrigation was performed in an efficient way. This must have been due to the fact that *two* Miller-Abbott tubes were introduced. It is evident that in nauseated,

uremic patients such a procedure would be impossible. It seems probable that the authors only succeeded because their patient was comatose and did not resist too much against this heroic treatment. Nevertheless, even this patient pulled out both tubes twice and vomited the proximal tube on one occasion.

Summarizing it seems certain that colonic irrigation is useless as far as the treatment of uremia is concerned. It still has not been proved whether intestinal irrigation of the small intestine has a future in the treatment of uremia and anuria. Table XII illustrates that by this method relatively small amounts of urea are removed. It is probably significant that in the two best cases reported the blood urea nitrogen decreased considerably but the creatinine content of the blood did not. Finally no satisfactory method for efficient intestinal irrigation has been devised yet and dangerous, even fatal complications have been caused by changes of the electrolyte content of the blood.

Exsanguinotransfusion

It has been known for many years that an uremic animal can be kept alive by exchange transfusion. An artery of the uremic animal is connected with the vein of the donor animal, a vein of the uremic animal with an artery of the donor. Formerly, paraffinated cannulae were used, nowadays heparin is injected. This favorable result is evidently due to the replacement of the blood of the uremic animal by normal blood (Nyiri,[46] Thalhimer,[14] Thalhimer, Solandt and Best [47]).

The same principle is used in the exsanguinotransfusion where the total mass of the patient's blood is replaced by an equivalent quantity of normal blood. This method has been used rather extensively in newborns suffering from erythroblastosis fetalis and lately by Bessis and Bernard[48] in adults suffering from leukemia. Very recently at the instigation of Bessis this method has been used to obtain improvement in the condition of uremic patients (Pasteur Vallery-Radot, Milliez and Bessis), and it has been successful in six of seven cases. To illustrate the difficulties connected with the exsanguinotransfusion the case treated by Tzanck and Dausset[49] may be mentioned (Table XIII). They treated a forty-year old patient who had taken 16 capsules each containing 0.5 grams of aspidium filicis maris and 0.05 grams of calomel. The patient, in order to hasten the expulsion of the taenia also took magnesium citrate and sodium bicarbonate. It is well known that it is dangerous

TABLE XIII—INTOXICATION WITH FILIX MAS AND MERCURY TREATED WITH EXSANGUINOTRANSFUSION[40]

Day	*Urinary Output in cc.*	*Blood Urea Nitrogen Mg.%*	*Blood Withdrawn*	*Injected*
7	150	163	4 L 11 gm. U+	3.25 L blood 1.2 L NaCl 0.9%
8			3.65 L 9.6 gm. U+	2.55 L blood 1.00 L NaCl 0.9%
9	175	150	6 L 15.8 gm. U+	4.2 L blood 1.7 glucose 5%
10		153	5 L 14.87 gm. U+	3.5 L blood 1.5 L glucose 5%
11	280	133	5 L 12. gm. U+	3.5 L blood 1.4 L glucose 5%
12	375	130	5.5 L 12.9 gm. U+	3.8 L blood 1.6 L glucose 5%
14	300	151	6 L 16.26 gm. U+	4.2 L blood 1.8 L glucose 5%
15	675			
16	3,000		6 L 9.9 gm. U+	4.2 L blood 1.8 L glucose 5%
		Total	41.5 L blood 103.2 gm. U+	29.2 L blood and 12.0 L diluting solution

to take alkaline compounds together with calomel because soluble toxic mercury compounds may be formed. On the seventh day of oliguria the urinary output was down to 150 cc.; the urea nitrogen of the blood up to 163 mg. per cent. Patient was somnolent and showed involuntary muscular contractions. On this day the first exsanguinotransfusion was performed.

Three and one-quarter liters of blood were injected diluted with 1.1 liters of normal saline. The transfusion lasted 19 hours and 11 grams of urea nitrogen were removed with the blood. The next day 3.65 liters of blood containing 9.6 grams of urea were extracted and 2.5 liters of blood diluted with 850 cc. of normal saline injected. The next day another 6 liters of blood containing 15.8 grams of urea were extracted and 4 liters of blood diluted with 1.3 liters of 5 per cent glucose were injected. The blood urea nitrogen remained about 150 mg. per cent. The daily diuresis did not exceed

175 cc. The next day the blood urea nitrogen was 150 mg. per cent, the patient was somnolent and had involuntary muscular contractions. A fourth exsanguinotransfusion was performed. Five liters of blood were withdrawn, containing 14.9 grams of urea which were replaced with the equivalent amount of diluted blood. This operation was repeated on the next two days, 5 liters and 5.5 liters of blood were withdrawn, containing 12 and 12.9 grams of urea respectively. The urea nitrogen of the blood diminished slightly to 132 mg. per cent, the urinary output went up to 280 cc. and 375 cc. per day. Two days later the blood urea nitrogen had increased again to 151 mg. per cent, the daily urinary output did not exceed 300 cc. Another exsanguinotransfusion was performed. With the blood 6.3 grams of urea were withdrawn and 6 liters of diluted blood were injected. Thanks to the injection of 200 mg. of heparin this and the following procedures lasted two to three hours each. Now the output improved to 675 cc. per day. Two days later the eighth and last exsanguinotransfusion of 6 liters was performed. After this operation diuresis set in with a vengeance and amounted to 3 and 4.5 liters per day. The urea concentration of the urine increased gradually. The blood urea nitrogen decreased accordingly and on the twentieth day it was 43 mg. per cent.

During a total of eight exsanguinotransfusions given over ten days, 41.15 liters of blood were withdrawn and 41 liters of fluid were injected, consisting of 29.2 liters of blood and 10.8 liters of saline or glucose. The urea eliminated by the exsanguinotransfusion together with the small amounts of urea eliminated in the urine amounted to 125.9 grams. In this way every day an average of 12.6 grams of urea were removed. This may be the reason why notwithstanding persistent extreme oliguria the blood urea nitrogen instead of increasing, remained at the same level or even diminished slightly.

The advocates of this method emphasize that it has definite advantages over the treatment with the artificial kidney, peritoneal dialysis, and intestinal irrigation (Table XIV). In contrast to the other procedures the exsanguinotransfusion removes all toxic substances whether dialyzable or not, improves the protein, water and electrolyte content of the plasma and does not cause hemolysis.

In addition the French authors are of the opinion that the large quantities of fresh blood injected do not represent a simple substitution treatment. They believe that the blood of the anuric patient, full of toxic retention products, inhibits the functions of different organs. The fresh blood permits a resuscitation of the functions not only of the kidney but also of the other organs as well. Injection of heparin, about 200 mg. per operation, permits shortening of the procedure to two to three hours. On the other hand, the procedure is extremely laborious and quite expensive unless there are many members of the family who are in a position to donate the many liters of blood which are necessary.

TABLE XIV

Replacement Transfusion	*Intraperitoneal dialysis*
1. Withdraws all toxic products including those which are non-dialyzable (hemoglobin, myohemoglobin).	Withdraws only dialyzable products.
2. Replaces the pathological blood by normal blood and re-establishes the normal equilibrium of the body fluids.	Withdraws not only pathological products but also certain useful dialyzable substances.
3. Incidents not serious; accidents caused by irregular agglutinins prevented by necessary precautions.	Peritonitis frequently noted, either of the plastic type by adhesions or of an infectious type (septicemias due to B. perfringens post abortum).
4. Can be repeated as often as needed.	Usually cannot be prolonged for more than 5 days. Difficult to repeat due to the formation of adhesions.
5. Disadvantages: large quantities of blood, sometimes of a rare type must be available. Heparinization necessary.	

There may however be instances where repeated exsanguinotransfusions will enable the anuric patient to survive until spontaneous diuresis sets in.

SUMMARY

The treatment of anuria in acute glomerulonephritis and in lower nephron nephrosis is of great practical importance. Acute glomerulonephritis has a tendency to heal spontaneously and in lower nephron nephrosis regeneration of the tubules always starts after ten days have elapsed. Therefore, everything must be done in order to assure survival of the patient for ten or fourteen days after the anuria has started.

The outstanding measure in the treatment of acute anuria consists of reduction of intake of fluids, electrolytes and proteins. In general, the daily fluid intake should not exceed 800 cc. which is sufficient to compensate for the loss of water via the respiratory tract and the skin. In case water is lost by diarrhea or vomiting the fluid intake should be increased. A diet of 800 cc. of fruit juice can be used at least for a

short time. It may be advisable to increase the caloric intake by ingestion of fat and carbohydrate. During hunger considerable amounts of protein are broken down which give rise to the formation of extra urea and other nitrogenous metabolites. For this reason, Borst has advocated a diet consisting of 200 grams of butter and 200 grams of sugar. This diet might well be helpful were it not that for most of the patients the mixture is highly unpalatable. In nearly all cases of anuria intravenous injections of large quantities of fluids and salts are contraindicated. In hypochloremia sodium chloride should be given, in acidosis bicarbonate.

It seems that in most cases of acute anuria these measures will be sufficient to keep the patient alive. Occasionally more drastic measures may be necessary. The latter is often the case in patients with anuria who have been treated with large amounts of fluid and salts. Under these circumstances, the artificial kidney, peritoneal dialysis, intestinal irrigation and exsanguinotransfusion have been used, occasionally with success. All these drastic methods have their advantages and disadvantages and should only be employed if strict indications exist.

At the time of this writing eleven uremic patients have recovered after use of the artificial kidney, twenty-one after peritoneal dialysis. No method is available which permits a satisfactory intestinal irrigation and prevents dangerous changes in the electrolyte content of the blood.

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THE PLACE OF ELECTROLYTE STUDIES IN SURGICAL PATIENTS*

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DURING the past generation surgery has made rapid strides as a result of concentrated attention to the problems of abnormal physiology in patients with surgical diseases. Better understanding of the control of shock, more adequate attention to water and electrolyte balance, and improved anesthesia have been more important than the concurrent advances in surgical technique in giving the surgeon relatively safe access to the contents of the thorax and the brain, the last two anatomical frontiers to be attacked in the evolution of modern surgery. As a result of this shift in emphasis, the surgeon is now called upon as never before to master the practical applications in surgical therapy of fundamental biochemical and physiological methods, particularly if he is unwilling, as are most well-trained surgeons, to turn over to his medical colleagues the nonoperative aspects of surgical care. The surgeon who is willing to relinquish his responsibility for pre- and postoperative care of his patients is in danger of becoming nothing more than a technician and merits no place in the company of scientific medicine. It must be realized that few surgeons are likely to possess the fundamental training in biochemistry and physiology to bring forward new contributions in this field of research. However, it is entirely appropriate and, in fact, essential that surgeons assume the responsibility for applying to their clinical problems the knowledge which has been gained in the preclinical laboratories and the parallel research efforts of their medical colleagues. The topic of the present symposium provides a particularly apt illustration of this thesis. Surgeons must share to a large extent the indebtedness which all fields of medicine owe to Folin, Gamble, Peters, Van Slyke, Darrow, and all of their disciples. The work of these individuals laid the foundation for our present understanding of the prob-

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lems of fluid and electrolyte balance and no discussion of this subject could be complete without frequent references to their work. However, many of the data which they have recorded, and many of the interpretations based upon these data, are concerned particularly with diseases more frequently encountered by the internist or pediatrician than by the surgeon, and in order to develop the rules of management of conditions of a peculiarly surgical nature, it has been necessary that some surgeons should themselves participate in studies in this general field. Significant contributions in this field have been made by a number of surgeons, including Coller and Maddock, Moore, Moyer, Evans, and Abbott, all of whom are still carrying on active work.

Certain general differences could be pointed out between the problems encountered in surgery and those encountered in the fields of internal medicine and pediatrics. In the first place, the surgeon is more frequently confronted with rapid external losses of body fluids and the resulting intercompartmental shifts between circulating blood, extracellular space, and intracellular fluid compartments. Although most surgical patients enter the hospital with fairly normal kidney function, adequate at least for the prevention of shifts in the hydrogen ion concentration of the blood such as might occur in ordinary degrees of dehydration, the surgical patient is subject to transient disturbances in renal function due to trauma and anesthesia. Very possibly the largest number of problems for the surgeon derive from his dependence upon parenteral fluid therapy at some time during the course of management of every patient undergoing a major operative procedure. A not insignificant proportion of his difficulty in this area may in fact be a *result* of the fluid replacement therapy which he employs, particularly if a selection of fluid has not been based on adequate knowledge of the nature of the existing imbalance. The surgeon must constantly be concerned with the effects of trauma and of operative procedures and anesthesia upon renal function and must recognize that the consequences of over-administration of salt and water during periods of renal dysfunction are just as dangerous as the state of dehydration itself. Edema of the soft tissues and especially of the bowel and the lung is notoriously productive of difficulties for the surgeon, and in the final analysis may account for a significant proportion of postoperative deaths. In recently reviewing a series of case histories of patients dying after radical surgical procedures on the pancreas and duodenum, we

have been impressed with the large number who displayed evidences of improper management in respect to salt and water metabolism. Furthermore, during the past two years, with better attention being given to the avoidance of excessive salt and water replacement, we have been gratified to observe a sharp reduction in the operative mortality from these procedures. We may say without hesitation that the surgeon who is ambitious to maintain his mortality at an irreducible minimum must be just as much concerned with the necessity for physiological fluid replacement therapy as with the technical details of the surgical procedure itself.

The clinical problems of electrolyte balance in surgery fall into three main categories; first, the pre-operative correction of dehydration and electrolyte losses due to the patient's disease. In this category are pyloric stenosis, intestinal obstruction, peritonitis, pancreatitis, and severe burns. Second, the prevention and treatment of imbalance due to the operative procedure and its immediate sequelae. As examples of this, one might cite the ileostomy, the biliary or pancreatic fistula, and the employment of tube drainage of any portion of the intestinal tract. Finally, there is the group of problems mentioned above which derive from improper fluid replacement therapy. The significance of problems in this last category has only recently begun to be appreciated and will receive special emphasis in this discussion.

During the past eighteen months we have maintained on our surgical service a segregated five-bed metabolism ward which is adequately staffed with nurses and technicians so as to permit complete nitrogen and electrolyte balance studies directed toward the solution of some of the major problems in pre- and postoperative care. The original data presented in this paper are a product of the combined efforts of the team of workers assigned to this unit. One of us (H.T.R.) has been particularly concerned with the application to surgical problems of recent knowledge as to the significance of sodium and potassium shifts following periods of dehydration. This work has been made possible by the availability of an accurate flame photometer[1, 2] for direct measurement of sodium and potassium in body fluids, and the work here reported would not have been possible without access to this instrument. The time will undoubtedly come when this instrument will be an indispensable item of equipment in every clinical laboratory. Many of the previous errors in the assessment of the patient's electrolyte status have

been due to almost exclusive reliance upon measurements of chloride and bicarbonate concentrations. Even application of the formulas for indirect calculations of the total base are subject to substantial errors and at the same time fail to give any representation as to the absolute amounts of sodium and potassium in extracellular fluid. Obtained primarily as a research instrument, the flame photometer has now become so essential to the day-to-day management of our regular patients that it has been necessary to install a second instrument for routine laboratory use.

In presenting this subject no apologies are offered for employing the terminology which has been widely popularized by Gamble and other investigators cited above.[3] In this terminology the concentrations of the various electrolytes are expressed in terms of milliequivalents per liter rather than in terms of milligrams per 100 cc. of fluid. A very brief discussion of the chemical background for this terminology may not be amiss before proceeding with this discussion. Turning back our none-too-facile memories to the college course in inorganic chemistry we may recall that a molar solution is one comprising the molecular weight of a compound in a liter of water. A milliequivalent of an ion is 1/1000 of its atomic weight, so that a milliequivalent of the positively charged sodium ion is 23 milligrams. This combines in a liter with a similarly calculated figure for the negative chloride ion, which is 35.5 mgms. to make a total of 58.5 mgms. of NaCl, or a millimolar solution. In discussing concentrations of the various electrolytes, their relationship, or equivalence to one another, is of paramount importance because in the plasma of a normal individual, the sum of the positively charged ions bears a constant and approximately equal relationship to the sum of the negatively charged ions. Variations in this relationship can occur only in conditions of uncompensated acidosis or alkalosis. When chloride ions are lost by vomiting hydrochloric acid, the deficiency of chloride in the extracellular fluid is met by an increase in HCO_3 ions to exactly the same number. Transposition of concentrations as milligrams per cent to milliequivalents per liter may be made by the employment of formulae derived for each electrolyte from its atomic weight and valence and in making the transition from one system to another, it may frequently be necessary to apply such calculations. It is realized that the use of this terminology has not yet become wide-spread among surgeons, but application of newer knowledge in this field makes it

imperative that surgeons start thinking in these terms. Relations of ions in equilibrium with one another is fundamental to intelligent management of electrolyte studies, and it is impossible to move about in this field of knowledge without the use of modern tools of terminology. The time has come when editors of surgical publications and books should require the expression of electrolyte values in the terms of milliequivalents and millimoles per liter. The change would be no more complicated than that which has been successfully accomplished in transferring from the apothecary's system to the metric system in gravimetric and volumetric expressions.

There is no problem in surgery in which electrolyte studies are more essential than that of the dehydrated patient. However, when a patient comes in with dehydration due to prolonged vomiting, diarrhea, a severe burn, or water deprivation from any cause, the first step is necessarily to establish the diagnosis, but correction of the dehydration should not be delayed while diagnostic studies are being carried out. It is especially important to deal with dehydration promptly if it seems probable that an operative procedure will be required to correct the patient's disease, because dehydrated individuals are notoriously poor-risk subjects and are extremely vulnerable to anesthesia, blood loss, and shock. The dehydrated patient has a reduced blood volume, and a reduced interstitial fluid reserve together with an increased viscosity of the blood, so that the volume flow of blood through the tissues and vital organs is already compromised, and if one adds to this the consequences of anesthesia, transient hypotension, and further reduction in blood volume, the outcome is costly. However, the type of fluid replacement needed and the amount of fluid required will vary significantly according to the degree of dehydration and the source of the water loss. Figure 1, taken from an article of Dr. William Abbott,[4] shows diagramatically the volumes of fluid normally available for external loss at different levels of the gastro-intestinal tract. However, the composition of gastro-intestinal secretions varies at the different levels of the tract, and it makes a big difference whether the dehydration is due to pyloric obstruction, when acid gastric secretions are predominant in the fluid-loss, or whether the lesion is a low intestinal obstruction, where the predominant portion of lost fluid is the alkaline secretion of the small bowel. Also the relative amounts of sodium, potassium, chloride, and bicarbonate lost will vary according to the

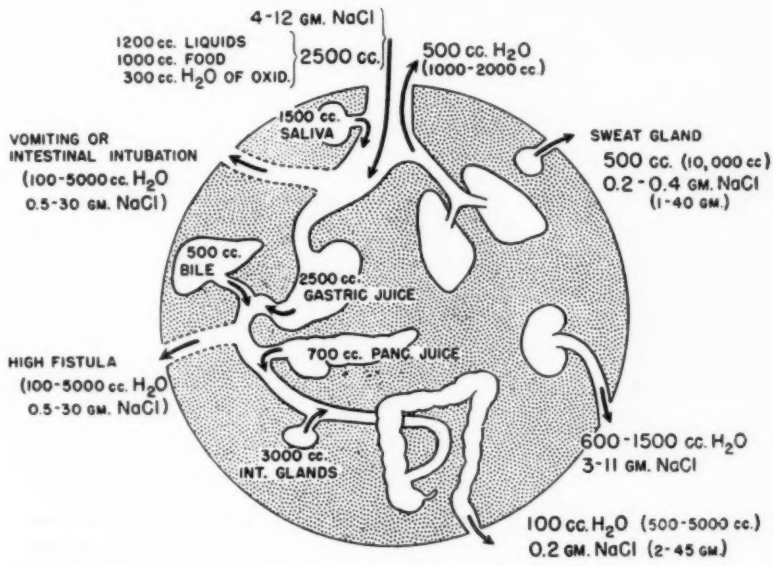

Fig. 1—Quantitative aspects of fluid and salt exchange in health and disease. (Abnormal losses indicated in parenthesis) From Abbott, W. E., Am. J. M. Sc., 211:232, Feb. 1946.[4]

route of the loss. Table I depicts the composition of fluids withdrawn at different levels of the gastro-intestinal tract. The average values as we have found them are shown together with the extreme range of values which we have encountered in the course of an eighteen-month study of surgical patients. One must guard against assignment of absolute significance to any of these figures because a given lesion rarely drains fluid exclusively from one segment of the gastro-intestinal tract; for example, in an obstructing lesion of the upper jejunum, the vomitus will contain considerable amounts of succus entericus, bile, and pancreatic juice, along with gastric juice. It therefore becomes essential in proper clinical management of such patients to determine the actual concentrations of electrolytes both in the plasma and in the vomitus or other drainage fluid and in the urine as well. Still further information may be obtained through the hematocrit reading, through measurement of the hourly volume flow of urine, and through determination of the plasma volume by the Evans blue-dye technique. The latter determination will be of less value as an absolute depiction of the

TABLE Ia—G.I. TRACT LOSSES M.Eq. PER LITER

	Na	*K*	*Cl*
GASTRIC	60.4	9.2	84.0
(Fasting)	9-116	0.5-32.5	7.8-154.5
SMALL BOWEL	111.3	4.6	104.2
(MA Suction)	82.-147.9	2.3-8.0	43-137
ILEOSTOMY	129.4	11.2	116.2
(Recent)	105.4-143.7	5.9-29.3	90-136.4
ILEOSTOMY (Adapted)	46	3.0	21.4
CAECOSTOMY	52.5	7.9	42.5
FORMED STOOL	<10	<10	<15

Electrolyte composition of gastro-intestinal fluids. (Upper figure indicates average; lower figure indicates range of observed values.)

TABLE Ib—BILE AND PANCREATIC FISTULAE

	Na	*K*	*Cl*
BILE	148.9	4.98	100.6
	131-164	2.6-12	89-117.6
PANCREAS	141.1	4.6	76.6
	113-153	2.6-7.4	54.1-95.2
URINE Normal	40-90	20-60	40-120
URINE Path.	0.5-312	5-166	5-210
TRANSUDATES	130-145	2.5-5.0	90-110

(Upper figure indicates average; lower figure indicates range of observed values.)

degree of reduction in plasma volume than as a baseline upon which to evaluate the adequacy of subsequent rehydration. Also the determination of the total red cell mass as calculated from hematocrit and plasma volume is of considerable value in determining the volume of blood transfusion which should be administered in conjunction with efforts at rehydration. Performance of these biochemical and physiological tests imposes a severe strain on the professional and technical staff of any hospital, but they have become an integral part of surgical management and success in dealing with severe problems of electrolyte imbalance in surgical patients cannot be attained simply by guess-work.

However, there are a few simple principles the observance of which will keep to a minimum the volume of laboratory work required and, more important, will help to keep the surgeon and his patient out of trouble. The balance of this paper will be devoted to emphasis of such points.

1. *Changes in the weight of the patient from day to day are a very useful index of over-all changes in hydration.* Although every effort should be made to measure and record accurately on the hospital chart the total volumes of both intake and output of everything which enters into daily fluid balance, there are often inaccuracies in such measurements. The total insensible loss and loss through perspiration can only be estimated very crudely, and drainage fluids and urine are frequently lost on dressings and bed clothes. The practice of early ambulation makes it possible to record daily patient weights even during the immediate postoperative period. A set of scales with a limit of accuracy of ½ pound is sufficient to obtain the relatively crude appraisal which is all one seeks by this method. In the case of a dehydrated patient it is possible to trace a successful effort at rehydration by progressive weight gain—which in a critical situation might be as much as 3 or 4 kilograms in 24 hours. On the other hand, the patient whose fluid regimen requires alteration because of the development of edema should undergo progressive weight loss if the new regimen has been well designed. Sudden changes in weight must always be accounted for, and unless other obvious causes exist, diurnal variations in excess of one half a kilogram are almost certainly attributable to loss or retention of body water. Attention then turns immediately toward meeting any pathological losses or gains by supplying more or less of whatever type of fluid is concerned.

2. *Every effort should be made to administer fluids by mouth in preference to parenteral routes* whenever the patient's disorder does not contraindicate use of the gastro-intestinal tract. The patient's actual needs for a substance such as salt are likely to be met more accurately by the dictates of his taste for salt than by the calculations of even the most solicitous surgeon, especially if accurate laboratory data are not continuously at hand. Furthermore, there is much less danger of having to meet the consequences of over-treatment if advantage is taken of the "intelligence" of the normally functioning gastro-intestinal tract. When we give sodium chloride solution as the sole source of electro-

lytes, we are failing to provide the significant amounts of potassium, magnesium, calcium, and other electrolytes present in natural foods, and, as will shortly be seen, knowledge is only beginning to catch up with the role of these inorganic ions in metabolism.

3. *Sodium chloride is a potentially dangerous drug, which is frequently indispensible in preserving life, and should be used with respect on both counts.* The normal intake of sodium chloride is generally given as about 2 to 4 grams per day and is approximately the amount excreted daily by the kidneys. This would be equivalent to 250 to 500 cc. of the usual so-called "normal saline" solution. Only under severe conditions of exposure to heat, such as might be encountered in a South African diamond mine, would the physiological requirement of salt for a healthy individual rise to equal the amounts which are often given to surgical patients in 2 or 3 liters of saline solution. Where there is an inability of the kidneys to retain sodium, as in Addison's disease, or where there is an extensive external loss of body fluids which contain sodium, the requirements of NaCl replacement are necessarily increased correspondingly and might even equal the quantities cited.

Specifications of proper requirements of sodium chloride for surgical patients have followed several swings of the pendulum between the extremes of inadequate and excessive salt therapy. However, much of the difficulty which surgeons have encountered has been due to over-reliance on sodium chloride alone in meeting electrolyte deficits. In 1938 Coller and his associates[5,6] proposed that hypochloremic patients should be given 0.5 gm. of salt per kilogram of body weight for each 100 mgms. that the plasma chloride level needs to be raised to reach the level of 560 mgms. per cent. Application of this rule might call for the administration of as much as 70 gms. of sodium chloride to a large individual who had a reduction of plasma chloride to a level of 360 mgms. per cent. The authors recognized that the need for sodium might not be identical with the need for chloride but stated that the normal kidney was perfectly capable of excreting sodium chloride given in excess of that actually required and that therefore it was desirable to err on the side of too much rather than of too little. However, in 1944 Coller[7] and his associates published an article on postoperative salt intolerance in which they retracted this clinical rule for salt administration and called attention to the inability of the kidney to excrete sodium chloride during the immediate postoperative

period. They reported a number of cases in which the application of their previously enunciated rule had resulted in serious over-hydration. Coller's new recommendation was that no saline solution of any kind be given during the day of operation and during the subsequent two postoperative days. It was further recommended that if a significant loss of extracellular fluid occurred during this three-day period, it should be replaced with 0.5 per cent sodium chloride solution containing 5 per cent glucose. It was his impression that at least 48 hours were required for the recovery of the kidney from the effects of the operative procedure and that during this interval great care should be exercised in the administration of salt. Administration of sodium chloride above that so required seemed prone to predispose to atelectasis, pulmonary edema, and intestinal dysfunction. Attention was called to the importance of careful observation of the physiological response of the dehydrated patient to test doses of salt solution rather than to reliance exclusively on laboratory determinations of the various electrolytes and other plasma constituents. Further emphasis on the significance of postoperative salt retention was supplied by Van Slyke and his colleagues[8] who pointed out that significant changes in renal function took place following periods of reduced renal blood flow such as might occur during the course of any major surgical procedure. Numerous students of the physiological mechanisms of cardiac failure have emphasized the effect of altered renal blood flow on salt retention and have even gone so far as to suggest that the edema of cardiac failure is due to renal dysfunction rather than to simple dynamic considerations, as had been previously assumed. Borst[9] suggests that salt retention by the kidney may be a protective mechanism designed to assist in the maintenance of plasma volume in shock and hemorrhage or in any situation in which cardiac output is decreased, a view similar to that originally held by Starling. Our own observations have confirmed the presence of marked impairment of renal blood flow during operative procedures and the subsequent tendency toward transient retention of sodium. One recent patient of ours excreted only traces of sodium for a period of 11 days during which the volume output of water and of chloride ion was approximately normal. The plasma sodium level was kept within normal limits only by rigidly excluding all sodium chloride from her fluid regimen. This case illustrates an important limitation of the Fantus test for urine chloride, as recently

TABLE II—COMPOSITION OF PARENTERAL FLUIDS
M.Eq. Per Liter

	Na	*K*	*Cl*	*Effective HCO_3*
0.85% NaCl	146	0	146	0
.9 % NaCl	154	0	154	0
110 Na+30 K	110	30	140	0
1/6M Na Lactate	166	0	0	166
NH_4Cl*	0	0	100	0
Dextrose+KCl**	0	30	30	0
Darrow's Solution	120	35	105	50
Hartman's Solution***	136	5.3	112	33
Dextrose in Water	0	0	0	0
Amigen	34	....	34	....
NORMAL Plasma	140.8	4.28	103	27.6

* 0.5% NH_4Cl+5% Dext.
** 0.223% KCl+5% Dext.
*** 3.6 meq/1Ca++

Electrolyte content of certain parenteral fluids compared with that of normal plasma.

repopularized by Van Slyke and Evans.[10] It is important to call attention to the fact that the kidney may retain sodium and yet excrete chloride in nearly normal amounts, so that the patient's need for sodium may be quantitatively quite different from his need for chloride ion. The results of a test for chloride in the urine may not reflect the condition of sodium balance. Our choice of fluids for electrolyte replacement must therefore be sufficiently flexible to separate these two requirements as necessary in special circumstances. Except when confronted with the problem of correcting or preventing an obvious deficit in body water, a patient should not be given more than his normal 2 to 4 gms. of salt a day or 250 to 500 cc. of saline. Additional requirements of fluid should be met with 5 per cent dextrose in water and additional amounts of salt should be given only upon clearcut indications therefor.

Electrolyte losses from the different levels of the gastro-intestinal tract may be compensated by the administration of fluids better designed than plain sodium chloride solution to meet the deficiencies likely to occur. In Table I is shown how the electrolyte composition of the various gastro-intestinal secretions varies in respect to sodium.

TABLE III—PARENTERAL FLUID REPLACEMENT

	Dext./W	*Dext./Sal*	*M/6Na Lact.*
GASTRIC	33%	67%	.....
SMALL INT.	20%	70%	10%
ILEOSTOMY	10%	75%	15%
BILIARY	.....	67%	33%
PANCREATIC	.....	50%	50%

Above solutions employed for volume-for-volume replacement of calculated losses. Basal intake should be 1500-2500 cc. with not more than 500 cc. saline.

Formulae which approximate average requirements in volume for volume replacement of gastro-intestinal fluid losses.

Table II shows by contrast the composition of some of the fluids which are available from our own Solution Room. Of these, only Hartman's solution approximates the electrolyte composition of extracellular fluid (plasma). Since it is convenient to replace the volume of fluid lost with a similar volume of replacement solution, it is suggested that the formulae indicated in Table III be employed. All three solutions are readily available in any hospital. When compensating for losses from the stomach on a volume-for-volume basis, it is wise to dilute "normal" saline solution with dextrose in water. When the drainage is from the small bowel such a mixture contains too much chloride, and a small part of 1/6 molar sodium lactate is added. The proportions of lactate increase progressively in the cases of ileostomy drainage, biliary and pancreatic fistulae, respectively. Since the ratio of sodium to chloride loss in pancreatic fistulae is approximately two to one, the appropriate mixture for the latter is equal parts of sodium chloride and sodium lactate. If these mixtures are given in amounts equal to volume of loss and the basal fluid intake is provided as indicated on the table, it is possible to anticipate and avoid some of the most troublesome sequelae of dehydration and over-hydration.

An especial word of caution is needed in regard to salt therapy in patients with low plasma protein levels. The tendency of the hypoproteinemic individual to hold extra water in the extracellular fluid is particularly aggravated in the presence of normal or high levels of plasma sodium. Although hypoproteinemic edema can be partially

combatted by simply withholding salt and permitting the sodium level to fall, the ideal treatment is to administer a high protein diet, and to meet the immediate protein deficit with transfusions of blood, plasma, or albumin. Otherwise, correction of the edema is gained at the expense of a reduced circulating blood volume which is in itself a serious handicap to the patient. It is in the hypoproteinemic patients that improper salt therapy is most likely to result in severe gastro-intestinal and pulmonary edema with marked dysfunction of both systems. Rhoads and his colleagues[11] at the Memorial Hospital encountered intractable hypochloremia in hypoproteinemic subjects and were able to correct this hypochloremia with sodium chloride until steps had been taken to restore the plasma protein level to normal. The question may now be raised as to whether these workers were dealing with simple chloride deficits or whether they were in fact confronted with associated potassium deficiencies, a syndrome which we now suspect may be frequently encountered in surgery.

4. *Severe dehydration is associated with loss of intracellular potassium and the patient cannot be restored to health until this potassium deficit is corrected.* Several years ago Darrow[12] called attention to the potassium deficits existing in infants after severe diarrhea and demonstrated that rehydration of these infants must be accomplished with fluids containing potassium as well as sodium. Very substantial reduction in mortality occurred when a combined electrolyte solution was administered in comparison with the mortality rate in patients treated with sodium chloride alone. When the flame photometer became available to us eighteen months ago, we commenced a study of the implications of Darrow's work in surgical patients with results which have been of considerable interest to us. According to Darrow's balance studies, there is a considerable loss of intracellular potassium consequent to the withdrawal of intracellular water in patients whose dehydration has gone beyond simple depletion of extracellular fluid. If sodium chloride solution is then given in amounts sufficient to rehydrate the individual, sodium moves into the cell to replace the lost intracellular potassium. This tends to produce intracellular edema and probable interference with many of the normal metabolic functions of the cell. However, the intracellular sodium can in turn be displaced and returned to its normal extracellular position if sufficient amounts of potassium are given before irreversible changes have occurred. In surgical patients

who are sometimes dependent for many days on parenteral fluids, a potassium deficiency can apparently occur even without the appearance at any time of marked dehydration. This is due to the fact that excretion of potassium by the kidneys goes on at a fairly regular rate of 10 to 20 milliequivalents per liter even in the absence of any potassium intake, the source being entirely endogenous. When the patient's balance requirements of calories and proteins are not being met, as is usually the case in surgical patients, a portion of this potassium loss reflects the nitrogen losses due to tissue catabolism. Furthermore, potassium is present in significant amounts in gastro-intestinal secretions and is especially high in pancreatic juice and bile. Therefore, if a draining fistula is present or if the patient is on gastro-intestinal intubation drainage, such additional losses of potassium will be added to those in the urine. A normal diet would ordinarily contain more than enough potassium to offset such losses. However, the patient who continues to be dependent on parenteral fluids for several days will tend to build up a cumulative potassium deficit equal to many times the normal potassium content of extracellular fluid, a total of only about 50 milliequivalents.

Although the clinical picture of potassium deficiency cannot yet be clearly defined, these patients are usually markedly asthenic and listless and in extreme cases will show myasthenia similar to that occurring in familial periodic paralysis, another potassium deficiency syndrome. Many of these patients give evidence of intestinal and gastric atony, and they are unable to eat adequate amounts of food. Blood chloride levels are normal or low, blood sodium levels are normal or high, the bicarbonate concentrations vary reciprocally with the chloride but are usually high in spite of the co-existence of an acid urine.[13] We now recognize that many patients who formerly did badly after major operative procedures, and who eventually succumbed, were probably suffering from this basic disorder in metabolism. Fortunately, the response of patients with potassium deficiency to adequate potassium therapy is prompt and frequently dramatic. Immediately after commencement of replacement therapy with a solution containing 30 milliequivalents per liter of potassium chloride, the patient displays an improvement in strength and appetite and usually within three to four days there is a marked diuresis with the excretion of a considerable quantity of sodium, presumably that which had become displaced from

its unphysiological intracellular depot. We have seen several patients become transformed within five to seven days and restored to full health after having previously been in a condition where recovery was despaired of. Caution must always be exercised in administering potassium-containing solutions because of the dangerous consequences of inducing an increase in plasma potassium to levels as high as 9 or 10 milliequivalents per litter, when heart block may occur. However, we have not observed such consequences in the patients we have studied, even though we have given as much as 120 milliequivalents of potassium, or 9 grams, within a twenty-four hour period to patients in whom a serious demonstrated deficiency existed. The specific contraindications of intensive potassium therapy are in those states where blood potassium levels are already high—namely, acute dehydration, severe burns in acute phase, intestinal obstruction, severe renal insufficiency, and during the first twenty-four hours after a severely traumatizing operative procedure. In these conditions the administration of potassium becomes safer after steps toward rehydration have become effectively under way. It is possible that the inclusion of a small amount of potassium, say 5 milliequivalents per liter, or .04 per cent, in all parenteral fluids might be the best means of preventing the development of such severe deficiencies as, once developed, would require therapy with more concentrated potassium solutions. Certainly no danger would exist from giving potassium in such amounts even to patients who did not actually need this ion.

Although much work remains to be done in correlating changes in plasma potassium with changes in potassium content of red cells and muscle tissue, we can now express confidence that the ability to recognize and to deal effectively with this syndrome represents a substantial improvement in the postoperative care of surgical patients.

Summary

The management of problems of water and electrolyte balance is of major importance in surgery because of the numerous instances of dehydration with which the surgeon must deal, and because of the frequent necessity to rely on parenteral fluid therapy for fairly long periods. In meeting these problems the surgeon should be conversant with modern terminology on matters of water exchange and ion equivalents and must receive the benefit of adequate laboratory assis-

tance in following losses and gains of these metabolites. Special considerations of importance are: 1) the usefulness of studying day-to-day changes in weight; 2) the importance of employing natural routes of feeding whenever possible in preference to parenteral routes; 3) the dangers inherent in excessive therapy with sodium chloride, particularly during periods of postoperative and post-traumatic renal dysfunction; 4) the syndrome of potassium deficiency is likely to occur in patients who are given excessive sodium during treatment or prevention of severe dehydration, and in patients carried exclusively on potassium-free fluids for several days.

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SECTION ON MICROBIOLOGY

NOVEMBER 17, 1948

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The Rh Factor
General Significance and Methods of Study

Philip Levine

The Rh factor is important clinically because of its capacity to induce isoimmunization when Rh positive blood is administered to Rh negative individuals, either by transfusions,[1] intramuscular injections, or by fetal blood across the placenta.[2] It has now become a routine procedure to carry out Rh tests on all prospective donors, candidates for transfusion and pregnant women for the prevention of isoimmunization by transfusion and for the diagnosis of hemolytic disease of the fetus and newborn.

The Rh-Hr Factors: The complexity of the Rh factor was noted very early in the course of the initial studies on the path of hemolytic disease of the fetus and newborn.[2] It is now established that the blood of an Rh negative individual is not devoid of antigenic properties, but it has an agglutinable property known as Hr.[3] At least three Rh factors are now recognized, each of which is serologically and genetically associated with corresponding Hr factors.[4]

The incidence of positive and negative reactions with each of the three sets of Rh-Hr factors are given in Table I, along with other pertinent data.

As first shown by Levine, anti-C and anti-c give three types of reactions; a type of blood failing to react with both anti-C and anti-c does not exist. As anticipated by Fisher, the same relationship holds for both the D-d and E-e systems, and is in striking contrast to the scheme of the four blood groups. It is because of the analogy to the serological and genetic behavior of the M and N factors that the letters in "Rh" were reversed to yield the term "Hr." Accordingly, the three types of bloods correspond to the three genotypes and the blood containing both the Rh and its genetically related Hr factor is heterozygous, while the other two types are homozygous (see Table II).

In each of the three sets, the difference between the positive reaction for the one (Rh or Hr) and the negative for the other (Hr or Rh) gives the incidence of the type which is heterozygous for both. Thus, in the case of the D-d factors, 85 — 37 or 63 — 15 = 48 per cent.

The incidence of the three types and of

TABLE I—THE THREE Rh-Hr SERA

Incidence of Reactions (%), Incompatible Matings (%) and Antibodies Produced

	+	—	*Incompatible Matings* (+ % —)	*Antibodies*
Anti-D (anti-Rh_0)	85	15	13	Frequent
Anti-d (anti-Hr_0)	63	37	23	Very rare
Anti-C (anti-rh′)	73	27	20	Very rare*
Anti-c (anti-hr′)	80	20	16	Occasional
Anti-E (anti-rh″)	30	70	21	Occasional
Anti-e (anti-hr″)	97	3	3	Very rare

* Anti-C occurs more frequently along with anti-D, in which case it is produced by an Rh- individual.

TABLE II

	Anti-D	*Anti-d*	%	*Incidence of Gene*
DD (homozygous)	+	○	37	$\sqrt{37} = 6.1$
Dd (heterozygous)	+	+	48	
dd (homozygous)	○	+	15	$\sqrt{15} = 3.9$

the two genes which in their several combinations form the three types, are given in Table III.

Given the frequency of a positive or negative reaction with, e.g., anti-D serum, the values of the corresponding Hr reactions can be derived from the following simple formulae:

1. $D + d = 10$
2. $D + 3.9 = 10$
3. $D = 10 - 3.9 = 6.1$
4. $(D + d)^2 = (6.1 + 3.9)^2 = 37 + 48 + 15 = 100$

Similar calculations can be made for the C-c system in which the incidence of heterozygous types is 53 per cent and for E-e 27 per cent.

Of the six properties, the most important factor is D or Rh_0 and it is significant historically that this factor was described independently by Levine and Stetson in 1939 and Landsteiner and Wiener in 1940. The former workers dealt with an agglutinin of human origin and the antigenic stimulus was believed to be derived from the transplacental transfer of fetal blood while the latter workers demonstrated the heterogenetic relationship of a factor common to human and rhesus blood, and first used the term "Rh."

A statistical study revealed that the blood cells of about 93 per cent of all mothers who deliver infants suffering from hemolytic disease of the newborn fail to react with anti-D or anti-Rh_0, and subsequent studies showed that their serum contains anti-D antibodies (agglutinins or blocking antibodies).[2,5,6] Similarly, almost all patients suffering from intra-group transfusion reactions are Rh negative. It is for this reason that all screening tests are done with anti-D (anti-Rh_0) diagnostic sera. From the point of view of diagnosis of hemolytic disease of the newborn and prevention of isoimmuni-

TABLE III—THE CLINICALLY IMPORTANT EIGHT BLOOD TYPES

Serum A (anti-B)	o		o		+		+	
Serum B (anti-A)	o		+		o		+	
Group	O		A		B		AB	
Incidence in White Race	45		41		10		4	
	38	7	35	6	8.5	1.5	3.4	0.6
Reactions with anti-D: Rh	+	—	+	—	+	—	+	—

zation and intra-group transfusion accidents, it is important for the clinician to think in terms of eight instead of four blood groups. These relationships are shown in Table III.

Irrespective of the reactions with the five other Rh-Hr sera, the 85 per cent of individuals whose blood contains the Rh factor will be designated as Rh+ while the remaining 15 per cent will be referred to as Rh—. In contrast to the scheme of the four blood groups, the serum of the Rh— individual does not normally contain the corresponding antibody which must be produced in response to the antigenic stimulus of Rh+ blood.

The data in Table I indicate that the factor D is far more antigenic than the sum total of the five other antigens. Excluding the rare instance of isoimmunization by the A or B factors, one may state that the D factor is more antigenic by the value of $\frac{93}{7}$ or at least 13 times more so than all other Rh-Hr factors. This is all the more remarkable since four of the incompatible matings (d, E, C and c) occur more frequently than the mating incompatible for D.

The various types of Rh+ and Rh— individuals and their nomenclatures are given in order of their frequency in the white population.

Rh+	1.	DCe	Rh_1	34
	2.	DcE	Rh_1Rh_2	15
	3.	DCE	Rh_2	14
	4.	Dce	Rh_0	2
Rh—	5.	dce	rh	13.0
	6.	dCe	rh′	1.5
	7.	dcE	rh″	0.5
	8.	dCE	rh′rh″	Very rare

From the point of view of the clinician, the significance of these subtypes has been unnecessarily overemphasized because the only serum available for routine testing is anti-D, and the use of this one serum will detect at least 93 per cent of potential isoimmunization. For the protection of Rh— patients who may have produced anti-C as well as anti-D, suitable compatibility tests carried out with at least two random Rh— donors will be satisfactory. Should anti-C and anti-E sera also be available, it is necessary to test only the Rh—donors and to exclude types 6, 7 and 8 as donors for immunized Rh— patients. They may safely be used, at least once, for Rh— patients who have not yet been immunized.

The final analysis of Rh+ individuals may be confined to husbands of Rh— women in order to determine their genotype and to prevent transfusion reactions in those rare instances (about 7 per cent) in which Rh+ patients may have produced any one of the five other varieties of antibodies.

Unfortunately, the serum required for the direct determination of the genotype, anti-d (anti-Hr_0), occurs very rarely so that it is necessary to resort to indirect methods. In brief, these are based on the frequency of certain gene combinations in the general population. For example, an Rh+ individual of type Rh_1 is homozygous for the D factor if his blood fails to react with anti-c (anti-hr′). Thus, his genotype is represented as *DCe/DCe*. This method will fail if the rare combination *dCe* is contributed by one of his parents. Similarly, individuals of type Rh_1Rh_2 are most frequently formed from the common gene combination *DCe* (Rh_1) and *DcE* (Rh_2).

Since each contributes the gene *D*, he must be homozygous for factor D even though he is at the same time heterozygous for the clinically less important factor C.

If anti-d sera were generally available for the differentiation of genotypes *DD* and *Dd*, the role of the other two Rh-Hr systems (C-c and E-e) would become far less important, and their clinical application could then be limited to the analysis of the 7 per cent Rh+ mothers of affected infants or patients immunized by repeated transfusions.

Detection of Isoimmunization: Two main sorts of Rh-Hr antibodies are recognized; 1) those which directly agglutinate saline suspended cells, and 2) those which specifically unite with their corresponding antigen but fail to cause a visible reaction. As shown by Diamond and Wiener, the latter type of antibody will give direct agglutination if the test cells are suspended in serum or 20-30 per cent bovine albumin. Coombs[7] has shown that the specific union of antigen and antibody can be detected if the thoroughly washed cells are tested with an absorbed anti-human precipitin prepared in rabbits or other suitable animals. Clumping results from the specific reaction of the globulin-containing antibody on the surface of the red cells, which serves as antigen and the anti-human globulin present in the precipitin serum.

Great confusion has resulted from the arbitrary use of a variety of terms for the two sorts of antibodies. These are listed below:

Agglutinins	Agglutinoids (glutinins)
Saline agglutinins	Albumin agglutinins
Heat-labile antibodies	Heat-stable antibodies
Complete antibodies	Incomplete antibodies
Bivalent antibodies	Univalent antibodies
Early antibodies	Late antibodies
	Cryptagglutinoids
	Conglutinins
	Blocking antibodies
	Coating antibodies

Tests for the detection of isoimmunization should be carried out in all prenatal cases and for all candidates for transfusion, Rh+ as well as Rh—. The test blood should be so selected that all six Rh-Hr antigens are present, such as in a mixture of two group O bloods, types Rh_1 and Rh_2.

From a practical standpoint, two methods may be employed in routine tests—the slide test or the test tube. The former is suitable for small scale work or in urgent cases while the test tube method is preferable for simultaneous tests of numerous sera.

For the slide test, about 40-50 per cent suspension of the test blood such as is present in (dry) oxalated blood should be employed. One drop of the patient's serum is mixed with one or two small drops of the test blood on a slide which is placed on a warm illuminated glass surface and rocked slowly from side to side.

For the tube method, it is desirable to test all sera with both saline and albumin suspended test cells. A more dilute suspension may be used (2-5 per cent). Readings may be made at the end of 1 to 2 hours' incubation. The presence of agglutinins is indicated by reactions in both tubes while the so-called blocking antibody will react only with the albumin suspended cells.

After the readings with the saline suspended cells are completed, the Coombs test should be carried out to detect specific coating. Each of the tubes is filled with saline and the cells are then washed at least three times, after which two drops of a processed and standardized anti-human precipitin is added.

The results with the Coombs test will serve to confirm the findings with the direct reaction with albumin suspended cells. At the same time, it will aid in the detection of so-called prozones due to the presence of both agglutinins and blocking antibodies in the same serum or due to the presence of several varieties of blocking or incomplete antibodies.[8]

No suitable substitutes for bovine albumin have been found. The several hydrophilic colloids (pectin, acacia, gelatin, polyvinyl alcohol) have the unfavorable property of inducing very strong pseudo-agglutination.[9] In titration of blocking antibodies, it

is preferable to use pooled normal male serum as a diluent and albumin suspended cells. A combination of serum and albumin in these tests gives stronger reactions and higher titers than serum-serum mixtures or albumin-albumin mixtures.[10]

In the tests for detection of isoimmunization, 30 per cent albumin as the suspended medium for the test cells gives stronger reactions than 20 per cent albumin.[10] Furthermore, prozone-containing sera will give at least weak reactions in the qualitative test with the greater concentration of bovine albumin. The writer, however, prefers to use 20 per cent bovine albumin which is less viscous, provided that the Coombs reaction is carried out on the saline suspended cells in order to detect prozones.

Once an antibody is detected, additional tests are required to determine its potency (by titration), its specificity in tests with cells of known antigenic structure and its avidity. All attempts should be made to collect large quantities of sera containing potent antibodies of the several varieties which will be useful for routine Rh-Hr testing and for determination of genotypes. More recently, attempts have been made to immunize donors on a voluntary basis and to stimulate the formation of potent antibodies in already immunized mothers who may have been sterilized or do not plan further pregnancies.

The normal iso-antibodies, anti-A and anti-B in all anti-Rh or anti-Hr sera, must be removed either by absorption with suitable cells or preferably by the addition of soluble A and B substances.

The Coombs reaction is of greatest value in testing the cord or capillary blood in order to determine if the infant's red blood cells are specifically "coated" with maternal antibodies. Curiously enough, only blocking antibodies but not agglutinins can be demonstrated on the surface of the infant's red blood cells. Strongly coated red blood cells, as indicated by a powerful reaction with the Coombs reagent, will fail to react with anti-D agglutinins and have been erroneously diagnosed as Rh—. Actually, such blood is Rh+ and fails to react because all Rh receptors have been saturated for long periods in vivo with maternal antibodies.

No attempt will be made here to correlate the serological findings with the prognosis in the infant, except to state that the presence of a positive Coombs reaction on the cord cells of an immunized Rh— mother does not by itself constitute an indication for a replacement transfusion. In general, the severity of the condition of the infant depends upon the length of the period of intrauterine passage of antibodies into the fetal circulation. Unfortunately, little is known of the factors which influence the transfer of the several varieties of antibodies across the placental barrier.

Mechanism of Transplacental Transfer: Isoimmunization by the Rh factor in fetal blood assumes an importance altogether out of its proportion to the low incidence of hemolytic disease because it is the first example in any species of a new cause of fetal and neonatal morbidity attributable to a genetic difference involving a particular blood factor which has a normal incidence in any racial group. In this connection, it is of interest that the incidence of hemolytic disease is lower in negroes (about 5-8 per cent Rh—, instead of 15 per cent and almost never observed in Chinese, Japanese or American Indians (less than 1 per cent Rh—).[9]

It is assumed that in every normal pregnancy a few fetal red blood cells find their way into the maternal circulation, at least in sufficient quantity to induce isoimmunization. This passage probably begins in the latter half of the pregnancy when the fetal blood vessels gradually become larger and, over an ever-expanding surface area, gradually approach the maternal sinuses from which they are then separated by a single layer of syncytial cells, aside from the endothelium. Other favorable circumstances are the sluggish circulation in the maternal sinuses and the greater pressure in the fetal circulation due to heart action and further increased by active fetal movement in the latter half of the pregnancy.

It is significant that the Rh factor does not occur in a water-soluable form and it is probably limited to the red blood cells, only small quantities of which suffice for

antibody production. The important role of transplacental transfer of fetal red blood cells is illustrated in those instances in which the firstborn infant is affected. In the vast majority of these cases, the Rh— mother had previously been transfused and the appearance of antibodies in the latter third of the pregnancy can be interpreted only as the response to antigenic material received in the course of the pregnancy. These cases serve as examples of the specific anamnestic reaction since antibodies resulting from the transfusion of perhaps 10-15 years previously have since disappeared, but they reappear more rapidly in response to the same antigenic stimulus many years later.

Additional evidence to indicate that there is no need to assume gross pathologic lesions in the placenta will be found in the recent papers of Naeslund and Aren, Kline and Everett and Henderson.* It would indeed be remarkable if nature provided an absolutely perfect organ which, like a malignant cell, is endowed with such invasive properties and rapid proliferation that in the short space of 40 weeks it attains a surface area of 70-120 square feet essential for the nourishment of the fetus.

Undoubtedly, further studies will reveal many examples of isoimmunization by fetal blood in other species of animals, especially those characterized by a type of placenta which does not differ much from that in man. Curiously enough, the first successful demonstration was in horses in which there are four layers of tissue cells separating the two circulations. Because maternal antibodies of the mare do not pass into the fetus, the disease does not occur in utero but only after ingestion of colostrum which is so rich in antibodies.[12, 13]

* For references see Levine.[11(b)]

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Recent Views on the Genetics of the Rh-Hr Blood Factors

HERLUF H. STRANDSKOV

At least 6 Rh-Hr antigens are known to exist. Credit for their discovery must be given to Landsteiner and Wiener, Levine, Race, Mourant, and Diamond. Originally the 6 antigens were called Rh′, Hr′, Rh_0, Hr_0, Rh″ and Hr″. In 1944 the British investigators, Fisher and Race, introduced another set of names known as the CDE system. Because of its simplicity and descriptiveness this system is gaining favor with many investigators, not only in Europe but in the Americas as well. It is the system which I personally prefer, primarily because I teach large classes in human

genetics and find that it is much more readily accepted by students. I should, however, like to see the symbol Rh shown before the CDE letters whenever it might not otherwise be clear that reference is being made to an Rh antigen. The two sets of symbols which have been proposed are shown in Table I.

TABLE I—NAMES OF RH-HR ANTIGENS

Original names	*Fisher-Race symbols*
Rh′ and Hr′	Rh C and Rh c
Rh_0 and Hr_0	Rh D and Rh d
Rh″ and Hr″	Rh E and Rh e

As is indicated in Table I the 6 antigens exist in pairs. A member of a given pair may occur without the other member in the blood of a given individual, or in combination with the other one. Hence with respect to each pair, 3 blood types are possible. Each of the 3 possible blood types relative to a given pair may exist in combination with each of the 3 possible types of each of the 2 other pairs. Accordingly 27 Rh-Hr blood types are possible. These 27 types may be designated by the original names, by the letters of Fisher and Race or by a set of abbreviated symbols advocated by Wiener. As I have already indicated, I personally prefer the CDE system but with the symbol Rh appearing before the blood type if it is not immediately clear that reference is being made to an Rh type. The original names are cumbersome and the abbreviated names of Wiener are not descriptive. The latter may be perfectly acceptable as abbreviations in a particular laboratory but in my opinon they are not the most acceptable for general use. The CDE system corresponds closely to the A-B or M-N terminology in indicating exactly the antigen present in the blood.

Landsteiner and Wiener were the first to report on the inheritance of the Rh blood factor. This was in 1941 when only 1 antigen was known, namely the one now called Rh_0 or D. These two investigators

TABLE II—THE 27 RH-HR BLOOD TYPES

Blood type using the original names of the antigens	*Recommended names*
1. Rh′ Rh_0 Rh″	Rh CDE
2. Rh′ Rh_0 Rh″Hr″	Rh CDEe
3. Rh′ Rh_0 Hr″	Rh CDe
4. Rh′ Rh_0 Hr_0Rh″	Rh CDdE
5. Rh′ Rh_0 Hr_0 Rh″ Hr″	Rh CDdEe
6. Rh′ Rh_0 Hr_0 Hr″	Rh CDde
7. Rh′ Hr_0 Rh″	Rh CdE
8. Rh′ Hr_0 Rh″ Hr″	Rh CdEe
9. Rh′ Hr_0 Hr″	Rh Cde
10. Rh′ Hr′ Rh_0 Rh″	Rh CcDE
11. Rh′ Hr′ Rh_0 Rh″ Hr″	Rh CcDEe
12. Rh′ Hr′ Rh_0 Hr″	Rh CcDe
13. Rh′ Hr′ Rh_0 Hr_0 Rh″	Rh CcDdE
14. Rh′ Hr′ Rh_0 Hr_0 Rh″ Hr″	Rh CcDdEe
15. Rh′ Hr′ Rh_0 Hr_0 Hr″	Rh CcDde
16. Rh′ Hr′ Hr_0 Rh″	Rh CcdE
17. Rh′ Hr′ Hr_0 Rh″ Hr″	Rh CcdEe
18. Rh′ Hr′ Hr_0 Hr″	Rh Ccde
19. Hr′ Rh_0 Rh″	Rh cDE
20. Hr′ Rh_0 Rh″ Hr″	Rh cDEe
21. Hr′ Rh_0 Hr″	Rh cDe
22. Hr′ Rh_0 Hr_0 Rh″	Rh cDdE
23. Hr′ Rh_0 Hr_0 Rh″ Hr″	Rh cDdEe
24. Hr′ Rh_0 Hr_0 Hr″	Rh cDde
25. Hr′ Hr_0 Rh″	Rh cdE
26. Hr′ Hr_0 Rh″ Hr″	Rh cdEe
27. Hr′ Hr_0 Hr″	Rh cde

postulated that a dominant autosomal gene existed which was responsible for the then known antigen. As this hypothesis was originally presented an individual who was Rh positive (Rh+) was either homozygous dominant, Rh Rh, or heterozygous, Rh rh; whereas an individual who was Rh negative (Rh—) was homozygous recessive, rh rh. The genetic results expected according to this original hypothesis are shown in Table III.

When Levine discovered the first Hr antigen, now called Hr′ or c, it became apparent that the original hypothesis of a dominant and a recessive allele could no longer be defended, at least, not in its entirety, because the so-called recessive gene

TABLE III—LANDSTEINER-WIENER GENETIC HYPOTHESIS (1941)

Genes	*Genotypes*	*Phenotypes*
Rh	Rh Rh	Rh positive (Rh+)
rh	Rh rh	
	rh rh	Rh negative (Rh—)

Possible matings	*Phenotypic ratio expected*
1. Rh Rh x Rh Rh	1 Rh+
2. Rh Rh x Rh rh	1 Rh+
3. Rh Rh x rh rh	1 Rh+
4. Rh rh x Rh rh	¾ Rh+ : ¼ Rh—
5. Rh rh x rh rh	½ Rh+ : ½ Rh—
6. rh rh x rh rh	1 Rh—

was found to produce an antigen and found to be as dominant as its allele. In other words, no dominance or equal dominance was found to exist. The results relative to the inheritance of Rh′ and Hr′ might then have been presented as shown in Table IV.

TABLE IV—GENETIC HYPOTHESIS FOLLOWING DISCOVERY OF Hr′ (Rh c) BY LEVINE

Genes	*Genotypes*	*Phenotypes*
Rh^C	Rh^C Rh^C	Rh C
Rh^c	Rh^C Rh^c	Rh Cc
	Rh^c Rh^c	Rh c

Possible matings	*Phenotypic ratio expected*
1. Rh^CRh^C x Rh^CRh^C	1 Rh C
2. Rh^CRh^C x Rh^CRh^c	½ Rh C : ½ Rh Cc
3. Rh^CRh^C x Rh^cRh^c	1 Rh Cc
4. Rh^CRh^c x Rh^CRh^c	¼ Rh C : 2/4 Rh Cc : ¼ Rh c
5. Rh^CRh^c x Rh^cRh^c	½ Rh Cc : ½ Rh c
6. Rh^cRh^c x Rh^cRh^c	1 Rh c

In Table IV the two alleles are designated by the basic locus symbol Rh and distinguished by the superscripts C and c. This is in accordance with a proposal which will be presented later for the symbols of all Rh genes. It should be noted especially that the phenotypic ratios shown in Table IV differ in certain instances from those shown in Table III.

As the second, third and fourth antigens were reported by various investigators and shown to have a hereditary basis, Wiener postulated that the genes responsible for all of these variations occupied a single locus, in other words, that a series of multiple alleles existed. He now assumes at least 8 alleles.

In 1944 Fisher and Race proposed a 3 linked-loci hypothesis with crossing over occurring or having occurred between the 3 loci. According to this hypothesis 8 homologous chromosomes, each with a different gene combination, may exist in a population. Fisher and Race have given the 3 pairs of genes the same names as the corresponding antigens, namely, C and c, D and d, and E and e. In my opinion this usage is an unfortunate one. If the 3 locus hypothesis should prove to be the correct one it would be preferable to use Rh as the basic locus symbol for all three loci and the letters CDE as superscripts. One specific objection to the use of C as a locus symbol is that it has previously been used in mammalian genetics, including human, for a locus affecting skin pigmentation or skin color. The 8 possible gene combinations on chromosomes, which are postulated by the 3 locus hypothesis, are shown in Table V.

As additional antigens were discovered and shown to be inherited, Wiener proposed symbols for the additional alleles which he assumed to exist. These symbols he has changed from time to time but his most recent set is probably that shown in Table V. In the opinion of many investigators, even those who favor the 8 allele hypothesis, these gene symbols proposed and strongly defended by Wiener are not fortunate choices. Among the arguments advanced against them is that they are not descriptive or suggestive of the actions which the alleles produce. Furthermore, they are not

TABLE V—GENE SYMBOLS

8 allele hypothesis		3 locus hypothesis		
Gene symbols of 8 alleles		*8 Gene combinations on chromosomes*		
Wiener	*Strandskov*			
R^z	Rh^{CDE}	Rh^C	Rh^D	Rh^E
R^1	Rh^{CDe}	Rh^C	Rh^D	Rh^e
r^y	Rh^{CdE}	Rh^C	Rh^d	Rh^E
r'	Rh^{Cde}	Rh^C	Rh^d	Rh^e
R^2	Rh^{cDE}	Rh^c	Rh^D	Rh^E
R^o	Rh^{cDe}	Rh^c	Rh^D	Rh^e
r''	Rh^{cdE}	Rh^c	Rh^d	Rh^E
r	Rh^{cde}	Rh^c	Rh^d	Rh^e

easily presented in long hand or in print without error. For example, the prime superscript and number 1 superscript are easily mistaken. Finally the use of some capital letters and some lower case letters is not in accordance with accepted genetic rules when no dominance or equal dominance of the alleles exists. Because of the numerous objections to Wiener's symbols which have been advanced I have proposed the use of Rh as the basic locus symbol and C D E letters as superscripts. In the proposed system the superscripts indicate specifically the antigens produced by each gene, if the 8 allele hypothesis is the correct one. Nearly the only valid argument in favor of Wiener's symbols is that they have priority. This argument deserves considerable consideration but we must also remember that whatever system is adopted now will be used for all time to come. Hence any one system should not be adopted lightly.

According to the 8 allele hypothesis 36 genotypes are possible. However, only 27 phenotypes or blood types should be produced, because some of the genotypes give duplicate phenotypes. The 36 genotypes and 27 phenotypes using both Wiener's symbols and those proposed by me are presented in Table VI. Only 27 genotypes are possible according to the 3 locus hypothesis. These are not shown in Table VI because they may be read directly from the 27 phenotypes which are indicated by the use of the CDE system of letters.

Since two hypotheses have been proposed to account for the inheritance of Rh-Hr blood types, and neither one is universally accepted, attempts have been made to determine which one is the correct one. To do so is not easy, but several types of discriminating evidence are possible. I shall mention and discuss briefly three of these possible lines, namely: 1) serological evidence, 2) cross-over results, and 3) gene, genotypic and phenotypic frequency analyses.

The possibly discriminating serological evidence is of the following type. According to the 8 allele hypothesis each gene is capable of producing 3 different antigens, whereas according to the 3 locus hypothesis each gene is responsible only for a single antigen. If we examine the action of other human genes which are known to be responsible for the production of antigens we find that each gene is responsible for the production of only 1 antigen. This at least is true of the genes responsible for A-B and M-N antigens. This is the simplest relationship imaginable and, therefore, seemingly the most probable. It does not follow, however, that the other relationship is impossible, namely, that a single gene can effect the production of 3 separate antigens as the 8 allele hypothesis assumes. Thus it will be apparent that it is my opinion that the serological evidence favors the 3 locus hypothesis but not to the extent of ruling out the 8 locus hypothesis.

The possible genetic evidence which may discriminate between the two proposed hypotheses is, as I have already stated, of two major types, namely 1) cross-over evidence, and 2) evidence obtained from an analysis of gene, genotypic and phenotypic frequencies in populations.

Cross-over evidence should be obtainable from a study of mating results if the 3 locus hypothesis is the correct one. By cross-over results is meant evidence that genes have been exchanged between homologous chromosomes in meiosis. To illustrate the results expected if crossing over occurs, let us assume a female of blood type Rh CcDdEe and who received the gene or genes responsible for the antigens C, D and

TABLE VI—GENOTYPES AND PHENOTYPES EXPECTED ACCORDING TO 8 ALLELE HYPOTHESIS

Proposed symbols (Strandskov)		*Wiener symbols*	
Genotypes	*Phenotypes*	*Genotypes*	*Phenotypes*
1. Rh^{CDE} Rh^{CDE}	1. Rh CDE	1. R^zR^z	1. R_zR_z
2. Rh^{CDE} Rh^{CDe}	2. Rh CDEe	2. R^zR^1	2. R_zR_1
3. Rh^{CDe} Rh^{CDe}	3. Rh CDe	3. R^1R^1	3. R_1R_1
4. Rh^{CDE} Rh^{CdE}	4. Rh CDdE	4. R^zr^y	4. R_zr_y
5. Rh^{CDE} Rh^{Cde} or Rh^{CdE} Rh^{CDe}	5. Rh CDdEe	5. R^zr' or r^yR^1	5. R_zr'
6. Rh^{CDe} Rh^{Cde}	6. Rh CDde	6. R^1r'	6. R_1r'
7. Rh^{CdE} Rh^{CdE}	7. Rh CdE	7. r^yr^y	7. r_yr_y
8. Rh^{CdE} Rh^{Cde}	8. Rh CdEe	8. r^yr'	8. r_yr'
9. Rh^{Cde} Rh^{Cde}	9. Rh Cde	9. $r'r'$	9. $r'r'$
10. Rh^{CDE} Rh^{cDE}	10. Rh CcDE	10. R^zR^z	10. R_zR_z
11. Rh^{CDE} Rh^{cDe} or Rh^{CDe} Rh^{cDE}	11. Rh CcDEe	11. R^zR^o or R^1R^z	11. R_zR_0
12. Rh^{CDe} Rh^{cDe}	12. Rh CcDe	12. R^1R^o	12. R_1R_0
13. Rh^{CDE} Rh^{cdE} or Rh^{CdE} Rh^{cDE}	13. Rh CcDdE	13. R^zr'' or r^yR^z	13. R_zr''
14. Rh^{CDE} Rh^{cde} or Rh^{CDe} Rh^{cdE} or Rh^{CdE} Rh^{cDe} or Rh^{cDE} Rh^{Cde}	14. Rh CcDdEe	14. R^zr or R^1r'' or r^yR^o or R^zr'	14. Rr_zr
15. Rh^{CDe} Rh^{cde} or Rh^{cDe} Rh^{Cde}	15. Rh CcDde	15. R^1r or R^or'	15. R_1r
16. Rh^{CdE} Rh^{cdE}	16. Rh CcdE	16. r^yr''	16. r_yr''
17. Rh^{CdE} Rh^{cde} or Rh^{Cde} Rh^{cdE}	17. Rh CcdEe	17. r^yr or $r'r''$	17. r_yr
18. Rh^{Cde} Rh^{cde}	18. Rh Ccde	18. $r'r$	18. $r'r$
19. Rh^{cDE} Rh^{cDE}	19. Rh cDE	19. R^zR^z	19. R_zR_z
20. Rh^{cDE} Rh^{cDe}	20. Rh cDEe	20. R^zR^o	20. R_zR_0
21. Rh^{cDe} Rh^{cDe}	21. Rh cDe	21. R^oR^o	21. R_0R_0
22. Rh^{cDE} Rh^{cdE}	22. Rh cDdE	22. R^zr''	22. R_zr''
23. Rh^{cDE} Rh^{cde} or Rh^{cdE} Rh^{cDe}	23. Rh cDdEe	23. R^zr or $r''R^o$	23. R_zr
24. Rh^{cDe} Rh^{cde}	24. Rh cDde	24. R^or	24. R_0r
25. Rh^{cdE} Rh^{cdE}	25. Rh cdE	25. $r''r''$	25. $r''r''$
26. Rh^{cdE} Rh^{cde}	26. Rh cdEe	26. $r''r$	26. $r''r$
27. Rh^{cde} Rh^{cde}	27. Rh cde	27. rr	27. rr

E from one of her parents, and the gene or genes responsible for the antigens, c, d, and e from her other parent. Her genetic composition might then be represented as CDE/cde, without implying that either hypothesis is the correct one. Now if she should marry a man Rh cde who may be represented as cde/cde, and they should have children, then evidence of crossing over would exist if children were born with blood types Rh Ccde, Rh cDde, Rh cdEe as well as children of blood types Rh CcDdEe and Rh cde. If no crossing over occurred then only children of the two latter blood types should be born to such a set of parents.

A large number of parents and their children have been studied for evidence of crossing over but none has been found so far. Race (1948) states that he has tested

over 150 families without detecting crossing over. Wiener and others likewise have studied many families and have found no evidence of crossing over. Hence if crossing over does occur it must be relatively rare. Thus it is apparent that the family studies presented so far give negative evidence in favor of the 3 locus hypothesis and are in agreement with the 8 allele hypothesis. They, therefore, may be said to favor the latter hypothesis. Perhaps we should mention that a single child suggesting crossing-over would not be sufficient evidence for the 3 locus hypothesis. The single observed result might represent a mutation rather than a cross-over. Only if cross-overs on a fairly large scale are observed can the evidence be said to rule out the 8 allele hypothesis and establish the 3 locus hypothesis. Fisher has suggested that indirect evidence of crossing over exists from an analysis of combinations of antigens in populations but this line of evidence, at least as advanced so far, is not very convincing.

The evidence in favor of either of the two proposed hypotheses based on observed gene, genotypic and phenotypic frequencies in populations is somewhat complex and cannot be dealt with in detail here but perhaps we may outline a few of the more direct lines of reasoning. By examining fairly large numbers of individuals in several different populations Wiener has obtained evidence that observed phenotypic frequencies agree fairly closely with frequencies expected on the basis of the 8 allele hypothesis. The British workers have been interested in comparing observed gene combinations on chromosomes with expected frequencies. They do not, however, assume that crossing has occurred to the extent that the genes at the different loci have reached combination frequencies comparable to random assortment. This is usually what would be expected if crossing over had occurred at all between the 3 loci. Rife has recently tested the 3 locus hypothesis by examining phenotypic frequencies on the assumption that equilibrium gene combinations have been reached. He finds that the observed phenotypic frequencies do not agree with those expected when this assumption is made. In other words he concludes that observed phenotypic frequencies do not agree closely with those expected on the basis of a 3 locus hypothesis. It must be admitted, however, that the test as applied may not be completely valid without a consideration of the role of selection or the make up of the sample population which was tested. Nevertheless, it seems that the gene, genotypic and phenotypic frequency analyses made so far favor on the whole the 8 allele hypothesis of Wiener rather than the 3 locus hypothesis of Fisher and Race.

SUMMARY

The following statements appear permissible relative to the genetics of the Rh-Hr Blood Factors.

1. The Rh-Hr blood types are completely genetically determined, that is, there is always a complete correspondence between phenotype and genotype.

2. An individual does not have an Rh-Hr antigen in his blood which is not present in at least one of his parents.

3. Neither of the two genetic hypotheses proposed to account for the inheritance of the Rh-Hr blood types has been completely established at the present time. The majority of the evidence, however, appears to favor the 8 allele hypothesis of Wiener rather than the 3 locus hypothesis of Fisher and Race.

4. Despite the fact that neither genetic hypothesis has been established, Rh-Hr blood tests may be used as evidence in legal cases of disputed paternity or baby mix up. However, evidence based on mode of inheritance must not be used.

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Medicolegal Aspects of the Rh-Hr Blood Types

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It was originally intended to confine this paper to the medicolegal applications of the Rh-Hr types, but I find that in order to make the subject intelligible it is necessary first to review the serology and genetics of the A-B-O blood groups and the M-N types. While it is true that the Rh-Hr types are more complicated than the A-B-O groups or the M-N types, the principles involved are the same, and knowledge of the facts and of the problems relating to the A-B-O and M-N types facilitates mastering the Rh-Hr blood types. Also the supposed simplicity of the A-B-O groups and the M-N types has been overemphasized, because when *all* the facts known concerning these two systems are taken into account the situation is no longer simple. In view of the clinical importance of the Rh-Hr types, the physician is constantly confronted with all the intricacies of this system while he is not compelled to learn the more refined facts concerning the other systems. The serologist and the medicolegal expert, however, must know the facts in their entirety.

TABLE I—THE FOUR LANDSTEINER BLOOD GROUPS

Blood groups (Phenotypes)	*Reactions of blood cells with serums*	
	Anti-A	*Anti-B*
O	—	—
A	+	—
B	—	+
AB	+	+

In Table I we have summarized the reactions given by human red blood cells when tested with anti-A serum and anti-B serum, determining the four Landsteiner blood groups. This represents about all that the average physician is required to know concerning this subject, which accounts for its apparent simplicity. As will be pointed out shortly, however, the situation is actually far more complicated. At this point, it should be emphasized that for medicolegal

work all blood tests should be carried out at least in duplicate and preferably in quadruplicate, using as controls blood specimens of known groups and types. In the case of the blood groups, the reciprocal relationship between the iso-agglutinins in the serum and the agglutinogens in the red cells (Landsteiner's rule), except during the neonatal period, supplies an additional method of checking the worker's results, and tests for the agglutinin content of the serum are an essential part of the examination in order to further guarantee the accuracy of the results.

To account for the heredity of the blood groups, a number of theories were proposed, namely, inheritance by two independent pairs of allelic genes A-a and B-b (von Dungern and Hirszfeld); inheritance by two pairs of genes either completely or partly linked (Furuhata, Bauer); or inheritance by a series of multiple allelic genes (Bernstein). The existence of separate pairs of genes for A and B, A-a and B-b, was readily disproved by Bernstein because this would imply that the distribution of the four blood groups would satisfy the following relation: O x AB = A x B. Since in New York City, for example, the approximate frequencies of the four groups are group O, 40 per cent; group A, 40 per cent; group B, 15 per cent; and group AB, 5 per cent, it is obvious that the equation does not hold. On the other hand, the theory of multiple alleles was proved to be correct by studies on families as well as by statistical analysis of the distribution in the general population.

As suggested by Strandskov, the three allelic genes postulated by Bernstein may be designated as I^A, I^B, and I^O, respectively, and this conforms with the practice of geneticists to use a common base symbol for all genes of the same allelic series. The symbol "*I*" was selected as a base symbol to represent "isoagglutinogen." These designations also have the advantage that the symbols for the genes, which incidentally are printed in italics, are quite distinct from the symbols for the corresponding agglutinogens, so that there is no ambiguity between the designations of phenotypes and genotypes. It is clear that according to Bernstein's theory, corresponding to the four phenotypes there are six genotypes possible, as shown in Table II. Since here we are interested only in the medicolegal applications, the results of Bernstein's theory are summarized in the following two laws:

TABLE II—THE A-B-O PHENOTYPES AND GENOTYPES

Phenotypes	*Genotypes*
O	I^OI^O
A	I^AI^A and I^AI^O
B	I^BI^B and I^BI^O
AB	I^AI^B

1. The agglutinogens A and B cannot appear in the blood of a child unless present in the blood of one or both parents.

2. Group AB parents cannot have group O children, and group O parents cannot have group AB children.

As has been indicated above, however, the situation is actually far more complicated. Firstly, there exist variants of the agglutinogen A, the most important of which are designated as A_1 and A_2, respectively. Rarely, additional variants designated as A_3 and A_4 have been encountered, as well as variants which give reactions intermediate between A_1 and A_2. Similar variants of agglutinogen B probably exist also, but these are less sharply defined so that a satisfactory technique for demonstrating them has not yet been devised. Moreover, the blood group O is not merely characterized by the absence of agglutinogens A and B, but also by the presence in the red cells of a special agglutinogen O which is shared also by all bloods of subgroup A_2. Finally, there is also evidence for a property C shared by agglutinogen A_1 and B. Omitting the rare variants A_3, A_4, etc., these facts have been summarized in Table III. Because of limitations of space, it is not possible here to go into further details concerning the concepts presented in this table. It may be of interest, however, to summarize the medicolegal implications of the subgroups of A, according to the following two laws:

TABLE III—THE A-B-O SERIES OF GENES AND THE REACTIONS THEY DETERMINE

Genes	*Corresponding agglutinogen*	*Reactions with serums*				
		Anti-A_1	*Anti-A*	*Anti-B*	*Anti-C*	*Anti-O*
I^{A^1}	A_1	+	+	−	+	−
I^B	B	−	−	+	+	−
i^{A^2}	A_2	−	+	−	−	+
i^O	O	−	−	−	−	+

1. No child can belong to subgroup A_1 or subgroup A_1B unless one or both parents belong to one of these subgroups. For example, two parents both of subgroup A_2 cannot have a child of subgroup A_1.

2. Parents of subgroups A_1B cannot have children of subgroup A_2, and parents of subgroup A_2 cannot have children of subgroup A_1B.

In the ordinary medicolegal cases where the blood group tests are applied a man is accused by a woman of the paternity of her child born out of wedlock, and he denies the charge. If the man has been falsely accused, by blood tests it may be possible to demonstrate his innocence, namely, if the groups of the man, the mother and her child do not satisfy the laws enunciated above. Obviously, the mere fact that the blood groups correspond is no proof that the accused man is the father, so that the tests can only be used to exclude paternity or maternity. The chances of exclusion will vary with the distribution of the blood groups in the population, and, among Caucasians, a falsely accused man has about one chance in six of establishing his innocence. The A_1-A_2 subgroups add only 1 to 2 per cent of the chances of exclusion, and for this reason as well as certain technical serological difficulties are but rarely applied to the court room.

A substantial step forward resulted from the discovery of the M-N blood types by Landsteiner and Levine in 1927. The fundamental facts concerning these blood types are even simpler than those of the blood groups, as can be seen from Table IV. Except for certain rare exceptions, however, isoagglutinins for M and N do not exist in normal human serum, so that the antiserums are usually prepared by immunizing rabbits. As Landsteiner and Levine pointed out, the existence of only three M-N types is readily explained by postulating their hereditary transmission by a simple pair of allelic genes, L^M and L^N, the base symbol L being selected in honor of Landsteiner, the father of blood grouping. The genetic implications of the theory are readily worked out since there is a one-to-one correspondence between genotypes and phenotypes (*cf* Table V), and there is ample statistical evidence from the study of families to confirm the accuracy of the theory. For the purpose of this paper, however, it is sufficient to summarize the results of the theory in the following two laws:

1. The agglutinogens M and N cannot appear in the blood of a child unless present in the blood of one or both parents.

2. Type M parents cannot have type N children, and type N parents cannot have type M children.

TABLE IV—THE THREE M-N TYPES

Blood types (Phenotypes)	*Reactions of blood cells with serums*	
	Anti-M	*Anti-N*
M	+	−
N	−	+
MN	+	+

TABLE V—THE M-N PHENOTYPES AND GENOTYPES

Phenotypes	*Genotypes*
M	$L^M L^M$
N	$L^N L^N$
MN	$L^M L^N$

With the combined use of the M-N types and the A-B-O groups, the chances of excluding paternity where a false accusation has been made are raised from 16 per cent to approximately 30 per cent. It might be pointed out that the chances are not simply additive because of the existence of cases with a double exclusion by both tests simultaneously. Therefore, no matter how many new tests are added it is impossible ever to attain the ideal of 100 per cent exclusion.

Also, in the cases of the M-N types, the true situation is actually not as simple as implied in Tables IV and V. Variants of the agglutinogens M and N have been found, but these are rare and of little practical importance. Recently, however, an important step forward was made when Race and Sanger discovered in the serum of the mother of an erythroblastotic baby, an irregular isoagglutinogen for a new blood property designated by them as agglutinogen S. Statistical and family studies proved this agglutinogen to be related to the properties M and N. The existence of two variants in the agglutinogen M was therefore demonstrated, one with and one without the property S, and similarly two variants of the agglutinogen N were demonstrated to exist. Accordingly, instead of a pair of allelic genes, it is now necessary to postulate a series of a minimum of four allelic genes as shown in Table VI. The existence of a separate gene for the agglutinogen S, linked to or independent of the genes for the agglutinogens M and N, was readily disproved by the use of the product theorum referred to in connection with the A-B-O blood groups. For the present, however, the agglutinogen S is not available for medicolegal use because of the scarcity of the required antiserum; besides, more family studies will have to be done before the medicolegal application becomes justifiable.

The reader who has absorbed the facts concerning the A-B-O groups and the M-N types will have no difficulty in understanding and learning the scheme of the 8 Rh types shown in Table VII. It will be noticed that the symbol for agglutinogen Rh_0 is written with a large "R," while the symbols for agglutinogens rh′ and rh″ are written with a small "r." This is done in order to emphasize the special serological and genetic position of the agglutinogen Rh_0. Agglutinogen Rh_0 corresponds to the original rhesus factor discovered by Landsteiner and Wiener in 1937, with the aid of immune animal serums prepared by immunizing rabbits and guinea-pigs with the blood of rhesus monkeys. Nowadays the antiserums are usually produced by immunizing volunteer male type rh donors, or obtained from patients who have had hemolytic transfusion reactions or erythroblastotic babies. With the aid of the human antiserums it was demonstrated that three Rh factors exist instead of only one, designated as Rh_0, rh′, and rh″, respectively. The symbols Rh_0 is written with a capital "R" because Rh_0 is by far the most anti-

TABLE VI—THE M-N SERIES OF ALLELIC GENES

Genes	*Corresponding agglutinogens*	*Reactions with serums*		
		Anti-M	*Anti-N*	*Anti-S*
L^M	M	+	—	—
L^{Ms}	Ms	+	—	+
L^N	N	—	+	—
L^{Ns}	Ns	—	+	+

TABLE VII—THE EIGHT Rh BLOOD TYPES

Rh types (Phenotypes)	*Reactions with serums*		
	Anti-rh′	*Anti-rh″*	*Anti-Rh$_0$*
rh	—	—	—
rh′	+	—	—
rh″	—	+	—
rh′rh″	+	+	—
Rh$_0$	—	—	+
Rh$_1$	+	—	+
Rh$_2$	—	+	+
Rh$_1$Rh$_2$	+	+	+

TABLE VIII—THE Rh PHENOTYPES AND GENOTYPES

Phenotypes	*Genotypes*
rh	*rr*
rh′	*r′r′ and r′r*
rh″	*r″r″ and r″r*
rh′rh″	*r′r″*, *rʸr*, *rʸr′*, *rʸr″*, and *rʸrʸ*
Rh$_0$	*R^{0}R^0* and *R^{0}r*
Rh$_1$	*R^{1}R^1*, *R^{1}r′*, *R^{1}r*, *R^{1}R^0*, and *R^{0}r′*
Rh$_2$	*R^{2}R^2*, *R^{2}r″*, *R^{2}r*, *R^{2}R^0*, and *R^{0}r″*
Rh$_1$Rh$_2$	*R^{1}R^2*, *R^{1}r″*, *R^{2}r′*, *R^{z}r*, *R^{z}r′*, *R^{z}r″*, *R^{z}rʸ*, *R^{z}R^0*, *R^{z}R^1*, *R^{z}R^2*, *R^{z}R^z*, *R^{0}rʸ*, *R^{1}rʸ*, and *R^{2}rʸ*

genic and therefore the most common cause of clinical complications. In fact, in most clinical laboratories, the tests are usually confined to the use of anti-Rh$_0$ serum alone. For clinical purposes, individuals of any of the four types, rh, rh′, rh″, and rh′rh″ should be considered Rh negative (Rh$_0$ negative) because they can all be sensitized to the most important Rh factor, Rh$_0$. As blood donors for Rh-negative patients, however, only type rh (triple Rh negative) individuals should be used, in order to avoid reactions in patients sensitized against the rh′ or rh″ factor.

As soon as I had demonstrated the existence of eight Rh blood types, it was obvious to me that I had to consider all possible modes of inheritance of the Rh blood types just as in the case of the A-B-O blood groups. With the product theorum, referred to above, I disproved the existence of separate genes for each of the Rh factors, and it was then evident that the eight Rh types are inherited by a series of multiple allelic genes. Eight principal Rh genes are recognized at the present time, which are designated as *r*, *r′*, *r″*, *rʸ*, *R^0*, *R^1*, *R^2*, and *R^z*, respectively. It will be noticed that, as in the case of the A-B-O groups and the M-N types, care has been taken to use different symbols for the genes and the agglutinogens in order to avoid ambiguity. The base letter "*R*" stands for the rhesus factor, and in this case the symbols for the genes are even simpler than the symbols for the agglutinogens, in contrast to the situation for the A-B-O groups and the M-N types.

As shown in Table VIII, 36 genotypes are theoretically possible corresponding to the 8 Rh phenotypes. The number of recognizable phenotypes has been increased to 27 types by the discovery of the so-called Hr antiserums, of which three varieties exist corresponding to the three Rh antiserums. The Rh and Hr antiserums are reciprocally related like the M and N antiserums, so that the reactions determined by the 8 Rh genes can be summarized as shown in Table IX. With the aid of this table, the phenotype corresponding to any given genotype can readily be deduced, but for the sake of brevity the details are not reproduced here.

For medicolegal use, most experts have available antiserum of specificity Rh$_0$, rh′, rh″ and hr′, and a few also have antiserum of the specificity hr″. Antiserum of specificity Hr$_0$ is still not available for general use. The medicolegal implications of the Rh-Hr tests, using the first five named serums, can be summarized in the following rules.

1. Factors Rh$_0$, rh′, rh″, hr′, and hr″ cannot appear in the blood of a child unless present in the blood of one or both parents.

2. Parents of types Rh$_1$Rh$_1$ and rh′rh′

TABLE IX—THE Rh SERIES OF ALLELIC GENES

Genes			Reactions with Rh serums			Reactions with Hr serums		
Designations	*Approximate frequencies among N.Y.C. whites (per cent)*	*Corresponding agglutinogens*	*Anti-Rho*	*Anti-rh′*	*Anti-rh″*	*Anti-Hr_0*	*Anti-hr′*	*Anti-hr″*
r	38.0	rh	—	—	—	+	+	+
r′	1.4	rh′	—	+	—	+	—	+
r″	0.5	rh″	—	—	+	+	+	—
ry	.01	rh_y	—	+	+	+	—	—
R⁰	3.2	Rh_0	+	—	—	—	+	+
R¹	40.4	Rh_1	+	+	—	—	—	+
R²	16.4	Rh_2	+	—	+	—	+	—
Rz	0.1	Rh_z	+	+	+	—	—	—

cannot have children of types rh, Rh_0, Rh_2 or rh″, and parents of types rh, Rh_0, Rh_2 and rh″ cannot have children of types Rh_1Rh_1 or rh′rh′. Or, more simply, hr′-negative parents cannot have rh′-negative children, and rh′-negative parents cannot have hr′-negative children.

3. Parents of types Rh_2Rh_2 and rh″rh″ cannot have children of types rh, Rh_0, Rh_1, or rh′, and parents of types rh, Rh_0, Rh_1, and rh′ cannot have children of types Rh_2Rh_2 or rh″rh″. Or, more simply, hr″-negative parents cannot have rh″-negative children, and rh″-negative parents cannot have hr″-negative children. As may well be imagined, the discovery of the Rh-Hr blood types has resulted in a substantial increase in the chances of exclusion for a falsely accused man, namely, from 30 per cent to about 55 per cent.

During the past few years, the writer has accumulated a series of approximately 450 cases of disputed paternity in which complete tests were done for A-B-O, A_1A_2, M-N, and Rh-Hr, including the factors Rh_0, rh′, rh″, and hr′; more recently it has been possible to add also tests for factor hr″. Among these cases there were 31 in which paternity was excluded by the Rh-Hr tests. Among the 31 cases there were 14 in which paternity was also excluded by the A-B-O tests, or M-N tests, or both. Thus, in as many as 17 cases, it would not have been possible to exclude the falsely accused man were it not for the more recently discovered Rh-Hr tests.

Obviously, the potentialities of the subject are far from exhausted, and studies which are continuing at the present time all serve to confirm Landsteiner's concept of an individuality of human blood somewhat analogous to the individuality of fingerprints. For example, the situation in the Rh-Hr types is actually even more complicated, since variants of the Rh factor have been found, particularly of the factor rh′. Moreover, no mention has been made in this review of the agglutinogen P and its variants, or of other independent blood properties recently found by Race and his collaborators as well as by the present writer and his associates. While the complexities to the casual observer may appear more and more bewildering, to specialists in the field they become more and more fascinating. It seems apparent that a common plan underlies the entire complex superstructure, and one of the main purposes of future research should be to discover this basic plan and the function served by the existence of individual differences in human blood.

Appraisal of the Clinical Aspects of the Rh Factor

PETER VOGEL

My presentation will be limited to the appraisal of some of the clinical aspects as they affect hemolytic disease of the newborn or erythroblastosis foetalis. The data are not easy to evaluate or to compare. There is a considerable variability in hemolytic disease of the newborn and many of the milder cases may be overtreated. While there may be no substitute for clinical observation or judgment, in most cases of erythroblastosis foetalis, the laboratory facts are reliable and in most instances, have given us exact diagnostic information which has proved invaluable in treating the affected infants. There are a number of clinical findings in the newborn which make a diagnosis of erythroblastosis foetalis or hemolytic disease of the newborn, obvious to all. The signs of edema, jaundice, pallor and purpura, twitching, convulsions, and splenic and liver enlargements are all characteristic. All these symptoms and signs may be mild, moderate or severe. There may be a marked anemia with reticulocytosis and an increased number of nucleated red cells in the peripheral blood. However, the diagnosis, in at least half of the cases, can not be made too early on clinical grounds alone, and particularly with the mild cases, may not be made for several days or weeks. The diagnosis may be suspected at all times when the mother is found to be RH negative and prenatal tests reveal anti-RH antibodies in her serum. Many of the involved infants are not anemic at birth and appear altogether normal. Within several hours jaundice is detected, and some degree of anemia develops. In some of these babies the signs and symptoms develop very rapidly so that in 24 to 48 hours, the infant appears very listless, cries weakly, refuses feeding or vomits the little it takes. Overwhelming toxicity supervenes and in spite of all measures at this time the infant succumbs. This was the usual story before the days of prenatal RH testing. At the present time, the diagnosis should, in nearly all instances, be made or suspected before the baby is born. Prenatal testing of the mother with antibody tests, if necessary, should be performed so that there will be no delay in carrying out the appropriate treatment. There are a number of instances when the tests are incorrectly performed. Regardless of previous errors in RH testing, or failure to detect antibodies in the mother, the diagnosis can be definitely established serologically in a few minutes.

In the past 18 months the use of Coombs' reagent has given us miraculous and dramatic support. With the use of this serum, negative tests have ruled out hemolytic disease of the newborn due to RH or HR sensitization while positive tests indicated the presence of the disease. When it is not known whether agglutinins found in the mother have been left over from a previous pregnancy or transfusion, a negative Coombs' test rules out involvement of the infant. On the other hand, the infant may have been erroneously typed RH negative because the cells have been blocked by the antibody transmitted from the mother. Under these circumstances a positive Coombs' test establishes the diagnosis without question. In our series every involved infant had a positive Coombs' test whether the mother had an agglutinin in saline or in albumin.

It might be mentioned at this time that, as yet, we have no good method in detecting A and B sensitivity in the infant. We must depend to a great extent on the hematological and clinical findings. The anti-A and anti-B titers at times may be helpful. In this group, however, the infants, usually first born, recover spontaneously. Some may require a single transfusion or an injection of A and B substance; rarely is the A and B sensitization severe.

The prognosis of erythroblastosis foetalis at the present time is not as difficult to make as it has been in the past, provided however the infant is born alive and in fair condition. With the proper treatment nearly all infants survive and recover dramatically. Most of the difficulty arises in

trying to predict the outcome in a particular pregnancy. A rising titer is indicative of an involved infant, but there are numerous exceptions to these findings. Even with vast experience, one can not be too sure how severely an infant will be involved and whether premature delivery is indicated. We have had a number of women who have been told they would not have infants which could survive because of their high antibody titer and their homozygous RH positive husbands. These women, nevertheless, have been delivered of infants, who, following replacement transfusions, survived and developed normally. On the other hand, there are a number of instances when mothers with relatively low titers have had infants severely involved or even still born. Although there is some correlation between the titer in the mother and the severity of the involvement of the infant, there is still much to be desired for accurate prediction.

It has been mentioned by several authors, that if a woman highly sensitized to the RH factor waits for several years, the anti-RH antibodies in her serum may decrease or disappear and following this her chances of having an infant which would survive would be better. In our experience there seems to be no basis for such advice. We have found that one year or more following delivery, the antibody titer in albumin remains fairly constant. We have had an exceptional example where a woman was delivered of her last child at the age of thirty-five. There had been no transfusions in the interim. At ninety-four years of age, blood examination revealed her to be RH negative and fairly potent anti-RH antibodies in albumin were demonstrable. I am sure that there must be numerous examples throughout the country of sensitized women whose antibodies persist for decades and all that is necessary to cause further stimulation is another pregnancy or a transfusion. It would therefore seem proper, that if further pregnancies are contemplated in sensitized women, not to advise dalay.

A few years ago there was a great number of Cesarean sections performed on women who had involved infants. The indications were often not warranted, the results poor, occasionally with the loss of both mother and baby. Now the pendulum has swung the other way in most clinics, because of the poor results previously obtained. We feel, however, that there is a certain group of mothers whose only chance of having an infant which might survive would be to deliver the infant somewhat prematurely by Cesarean section. In considering this procedure all factors must be weighed carefully to assure the survival of the infant following appropriate treatment. This group is comprised of women who have had a previous baby with erythroblastosis which was still-born, or did not survive the first few days of life. In these cases, undue conservatism leads to loss of another infant which may have been saved. The results are not good, but it is sometimes the only chance of having an infant that will survive.

The treatment of hemolytic disease of the newborn is most important. While there have been a number of people who have become interested in the hematological and serological findings for the diagnosis of this disease, there are relatively few who have devoted themselves to properly carrying out the indicated therapeutic procedures. In even such a large city as New York, there are only a few centers where the infants with erythroblastosis foetalis can be properly handled. It would seem much better for a number of pediatricians to learn the serological tests, and their interpretation and the technique of replacement transfusion, rather than have to depend on a laboratory report and then upon others to carry out the treatment. In many instances, babies in poor condition are transported for many miles because of lack of knowledge and training in home institutions.

For a severely involved infant who requires a replacement transfusion our routine is more or less as follows:

A complete blood count is done. The hemoglobin and red cell levels are important in order to calculate the amount of blood the infant should receive in excess of that which is withdrawn. The weight of the baby has to be known in order to estimate the total volume of blood. The blood used should be preferably fresh, or no more than 24 hours old. If not fresh, it should be warmed to 37°C. to avoid shocking the

infant. The baby should be kept warm throughout the entire procedure, with oxygen therapy if necessary. In some institutions like the Childrens' Hospital in Boston, the entire procedure is carried out in a specially built crib. Most of these infants are in very poor condition and extreme care must be used in handling them. Recently the General Electric Company has manufactured a special washable electric blanket bunting for us. This can be regulated to any temperature desired. It should soon be available for use in smaller hospitals where the hot water bottles and electric pads are not only inadequate but at times dangerous.

The treatment of choice for an infant with erythroblastosis is replacement or exchange transfusion. In the severely involved babies it is the only treatment at present which gives good results.

It is entirely possible that some infants which received a replacement transfusion may have survived with the ordinary methods of repeated transfusions, as in the past, but from our experience we have no way of determining which infants may suddenly begin to hemolyze blood rapidly, and by the third or fourth day succumb in spite of the fact that hemoglobin and red cells are fairly normal. It would therefore seem safer to do an exchange transfusion even in the borderline cases.

The technique of exchange transfusion is fairly simple. There are a number of techniques, such as replacement by way of the femoral vein, the radial artery and saphenous vein, and exchange by way of the umbilical vein. All appear to give equally satisfactory results. The question as to the superiority of one method over another will not be discussed at this time.

The exchange by the way of the umbilical vein has been the procedure of choice in practically all our replacements. It is thought by some that after the first 24 hours the umbilical vein can not be used, but we have been able to use this method as long as 68 hours after birth, without any ill affects. One should be prepared to use any method which seems suitable in a particular case. A special plastic catheter is passed into the umbilical vein and about ⅕ to ¼ of the blood volume is removed before any blood is replaced. The infant is kept on the anemic side until the procedure is more than half finished. In small infants a good portion of the plasma is removed from the donor's blood so that a smaller volume may be given in order not to overload the baby's circulation. We give 450 to 750 cc. of RH negative blood. Small amounts of calcium gluconate (1 cc.) are given a few times during the transfusion to conteract the rapid withdrawal of the available calcium. No heparin is used. After the exchange the infant is placed in a Davidson bed with oxygen if necessary. A large dose of vitamin K is given and repeated in 12 hours, 25,000 units of penicillin, B.I.D. for three days, and clyses are given, if necessary.

When possible we endeavor to leave the infant with an adequate red cell volume containing less than 5 per cent of its own cells. These remaining RH positive cells can be handled by the infant without danger whether hemolysis be slow or rapid. The Coombs' reagent is the only accurate method of determining how efficient the replacement has been. The antibodies transferred from the mother remain for 3 to 6 weeks at the end of which time RH positive cells can again be demonstrated in the circulation. However, at this time, the Coombs' test is negative.

The donor's blood is gradually eliminated from the baby's circulation and if there are any anti-RH antibodies remaining up to the fourth or fifth week, all the positive cells formed up to this time are destroyed so that some of the infants may require only a simple transfusion. By the sixth to eighth week increased production of red cells begins with moderate reticulocytosis. By the ninth to tenth week the trend is definitely upward and by the third month the infant approaches the normal figures for this age period and from then on develops normally. In a small percentage of infants mental retardation is noted and signs of kernicterus are found. The condition occurs in only a small percentage of patients and the pathogenesis remains quite obscure in spite of many theories. There are a few other complications of the disease which time does not permit me to discuss.

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